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## BULLETIN

OF THE



# AGRICULTURAL EXPERIMENT STATION

OF

NEBRASKA.

### A DRY ROT OF THE IRISH POTATO TUBER.

BY E. MEAD WILCOX, GEORGE K. K. LINK, AND VENUS W. POOL.

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<sup>\*</sup> Resigned, to take effect February 1, 1913.

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28 plates. 15 text figures. 7 graphs. 1 map.



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#### INTRODUCTION.

The first specimens of the dry rot herein described were received by the department of Agricultural Botany during the winter of 1907-1908 from western Nebraska. A preliminary survey of the situation showed clearly the importance of this new rot, and an exhaustive investigation of the disease was outlined and has been continued to the present time. During the summers of 1909 and 1910 a branch laboratory was maintained at Alliance in quarters generously provided by the local authorities to whom we are under obligations for the facilities placed at our disposal. In this manner we were able to study the prevailing local conditions and to secure results quite impossible of attainment in a laboratory located at a great distance from the seat of the trouble.

In addition to the authors of the present publication the following persons have at various times been connected with this investigation: Roland Elisha Stone, George Herbert Coons, and Ethel Field. To these persons we here extend our thanks for their faithful and conscientious service. Much of this work would have been impossible without the generous support of the Legislature in providing special funds for the work of the department of Agricultural Botany, while the more technical investigations have been made possible thru the use of the Adams Fund. We wish further to express our thanks to a large number of potato growers in the Sand Hill and High Plains regions for their interest and assistance, while to E. W. Hunt we are indebted for the earlier specimens furnished and for much infor mation as to the field conditions secured thru the use of a conveyance provided thru the public spirit manifested by W. L. Newberry of Alliance. We are indebted to the Department of Geography for the map of Nebraska soil regions.

The present publication gives the results secured to date in our investigation of this disease. Certain other problems remain

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<sup>\*</sup> Resigned November 1, 1911. Responsibility for all statements in this bulletin rests with the first two authors.

unsolved and these are now receiving attention. Among these may here be mentioned the determination of the real nature of the apparent resistance of young tubers and the stems to the invasion of this fungus.

#### HISTORY AND DISTRIBUTION.

It seems probable that some form of tuber dry rot due to species of *Fusarium* occurred long before it attracted the attention of potato growers or was investigated by plant pathologists. We have included in the bibliography references to some of the earlier accounts of what appears to be the same type of dry rot, tho in most cases the data given are not sufficient to enable one to express a positive opinion as to the identity of the causal

organism.

One of the earliest published accounts of a tuber dry rot was by Clinton 1895. He described (p. 139) a "bundle blackening" in the following words: "This is a fungous trouble of stored potatoes which shows as small dots or lines a short distance from the surface. The fungus gains entrance probably after the potatoes are gathered through the dead stem, and proceeds from this through the bundles, causing them to turn black as the result of its attack. The fungus is quite similiar to the one causing the following trouble." He then described (p. 139) the disease which he called "Dry End Rot" as follows: "It affects all the tissues as it slowly advances forward, until, perhaps, the whole tuber is destroyed. As in the preceding case, the trouble begins at the stem end, the fungus gaining entrance after the rupture of the tuber from the plant." He believed that this latter disease was caused by Fusarium solani and recommended that only sound tubers be placed in storage and these kept (p. 140) "in a dry, cool place."

Price 1897 described (p. 926) from Texas the "Dry Rot (Fusarium solani)" as follows: "This disease appears on the tubers in the form of a dark brown spot which is sunken beneath the surface of the potato. The disease spreads more rapidly

if the tubers be kept moist."

Bessey 1899 may possibly have had this same disease in hand and if so this would be the first reference to the disease in Nebraska.

Rolfs 1901 referred (p. 26) to the "Irish Potato Summer Rot" and says: "There is a rot of the Irish potato that seems quite distinct from *Bacillus solanacearum*, but the exact cause does not seem to have been ascertained. There is a fungus resembling a *Fusarium* quite constantly present but it may not be the cause."

Smith and Swingle 1904 described a dry rot and wilt of

potatoes which they claimed was due to Fusarium oxysporum. Subsequent authors in their discussion of similar symptoms of potato diseases have largely been guided by and often have merely repeated, without verification, the conclusions of Smith and Swingle.

What appears to be the same disease is described by anon. 1906 from England under the name "Winter Rot" and is claimed to be due to Nectria solani—an organism often mentioned in the literature as the "perfect" stage of Fusarium solani. This author says (pp. 739-740): "It attacks stored potatoes, and is always present to some extent, but as a rule only reaches the proportions of an epidemic during hot, dry seasons. The tubers only are attacked, and inoculation, through spores present in the soil, takes place when the tubers are young; but, as a rule, there is no obvious disease present when the tubers are lifted, although the mycelium of the fungus is present in the tissues. During the following season the most perfect stage of the fungus, in the form of minute crimson-red points, develops on the skin of the diseased tubers."

Norton 1906 referred (p. 67) to a "dry-rot" as caused by Fusarium oxysporum in the following words: "The disease is indicated by the lighter colored and more or less rolled up leaves. When dug the potatoes may appear sound, but internally show black or brown streaks, and later are destroyed at least at one end by the dry rot."

Morse 1908 says (p. 2): "Another disease of the stem and tuber which is usually designated as the *Fusarium* dry rot caused by the fungus *Fusarium oxysporum* Schlecht, has been found for the first time in Maine during the past summer. It is well known that this disease, and it is probable that black-leg as well, is dis-

seminated by means of seed tubers from infected fields."

Pethybridge and Bowers 1908 have described a dry rot of the potato tuber from Ireland where they say it first appeared in 1902 on the "Snowdrop" potato. They say (p. 548): "They were fairly firm to the touch, but more or less shrivelled, with the skin contracted into wavy wrinkles. At various points on the tubers the skins were broken through by fungus pustules. These were whitish on the surface; but on gently rubbing them the base of the pustule was seen to be of a blue color. On standing for some time, especially in the light, the surface of the pustules changed from whitish to a salmon-pink; on the less well-illuminated undersides of the tubers and on those in the dark the pustules remained for a long time whitish blue."

They ascribed this rotting to the fungus Fusarium solani Saccardo and emphasized the relationship of the rotting to pre vious wounding of the tubers. From 80 to 86 per cent of rotted tubers were found to have been previously wounded to some extent.

Duggar 1909 in his discussion of the "Dry Rot of Potatoes" (p. 317) says: "It is very probable that many of the diseases described under the name of dry rot, end rot, bundle blighting," etc., are due to the fungus here discussed. Smith and Swingle have, by careful cultural and inoculation experiments, demonstrated the causal connection of a Fusarium with these types of disease, and they have taken as the name of the species here discussed the earliest described species of Fusarium associated with such diseases, namely the one given above, and they would regard as probably synonymous with this species half a dozen or more names subsequently applied to fungi described as producing more or less similar types of disease in the potato."

Lounsbury 1909 has described and illustrated a dry rot of the potato tuber found in Cape Colony as caused by Nectria solani. He says (p. 42): "The infection is introduced into the soil with diseased tubers and it remains there from season to season, so that a crop from perfectly healthy seed may get infected if grown on land that previously bore an affected crop. The disease generally enters at the stem end. The fungus develops most rapidly in the "vascular ring." From his reference to the "wilting" symptoms shown by affected vines and the last one of his figures it appears likely that he has confused the tuber dry rot and the Fusarium wilt referred to by various American plant pathologists.

Jack 1910 has described a gall of the potato tuber from Rhodesia due to a species of *Heterodera* with which is frequently associated a form of dry rot. He says (p. 1533): "Their presence in numbers is followed by decay in the tissues and frequently by the wilting of the infested plant. In the case of potatoes the infested tubers yield very readily to the attack of

the common dry rot (Fusarium solani)."

Morse 1910 describing "Fusarium Dry Rot" (pp. 6-7) says: "The first symptoms are nearly always at the stem end, in the form of a brownish or blackened ring a short distance below the surface. At this stage the tuber may appear perfectly sound and healthy externally. Later there is a general discoloration of the flesh and a decided shrinkage of the tuber. The skin becomes wrinkled around the stem end, and the tuber becomes very light and often nearly as hard as wood. Infected potatoes may appear perfectly sound when placed in storage, and come out as described above."

<sup>&</sup>quot;When this disease is suspected the stem end of the tuber

<sup>\*</sup> Probably refers to "bundle blackening."

should be cut off and examined for a darkened cinnamon-brown

discoloration or ring in the tissues when cutting seed."

Tidswell 1910 described and illustrated a "Dry Rot" of the potato from New South Wales which he says is caused by Fusarium solani, an organism which he states should more properly be called Fusarium oxysporum. The illustration would indicate that he had to do with a dry rot quite similar to the one herein described tho by references to wilting of the tops of the plants, blackening of the vascular region, and root infection it seems certain that he had confused one or more other diseases with the dry rot of the tuber.

Manns 1911 has described from Ohio a disease called by him "the Fusarium blight and dry rot," which he says is caused by Fusarium oxysporum. Both his illustrations and the text show that the form of potato wilt described by Smith and Swingle 1904 and the present dry rot have been confused or rather united under one common designation. A full discussion of

Manns' inoculation work will be found under Etiology.

Jones 1912 describes what he calls "Wilt and Dry Rot." His conception of the matter is clearly shown by the following statement (p. 4): "This is a fungus disease which attacks the roots causing the potato tops to wilt and die before full maturity. It may also invade the tubers at the stem end causing the flesh to blacken, and in bad cases leads to dry rot in winter storage, especially of the stem end of the tuber. It may be detected in the field by the wilting of the plants and in the tubers by cutting off a thin slice across the stem end, where the disease shows as a brownish black spot or ring where the vessels from the stem enter the flesh. This disease, as it occurs in the tuber, is likely to be confused with the other types of internal browning. The distinctive characters are that this disease causes the tops to wilt and die and that the internal blackening of the tuber increases during storage and may lead to dry rot."

#### ECONOMIC IMPORTANCE.

Potato growing has long been a prominent part of the agriculture of northwestern Nebraska. The early "homesteaders" introduced potato culture into this region on a small scale to supply local needs. With the coming of the railroads the industry made rapid strides and the Sand Hill and High Plains regions soon established an enviable reputation for the excellent quality of their potatoes.

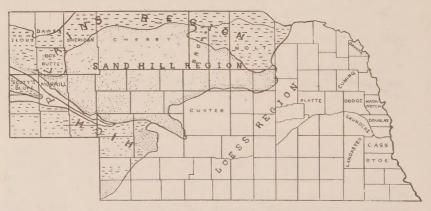
The full significance of the potato crop in this region may be seen from the following statistics taken from the last United

States Census Report covering the crop year 1909.

Table 1.—Acreage and yield of potatoes during 1909 in counties producing 100,000 or more bushels.

Counties	Acres	Bushels
Box Butte	7,020 *	303,918*
Brown	2,197*	174,862*
Cass	1,305	112,103
Cherry	2,978 *	150,105*
Cuming	1,280	125,608
Custer	2,410	159,308
Dakota	1,907	142,798
Dawes	2,322 *	124,782*
Dodge	1,406	121,574
Douglas	2,509	231,177
Holt	1,791	130,371
Lancaster	2,267	170,561
Morrill	1,535	107,156
Otoe	1,512	137,484
Platte	1,442	127,674
Saunders	1,363	123,315
Scott's Bluff	6,452	774,815
Sheridan	8,590*	668,309*
Sioux	2,688*	191,051*
Washington	1,251	104,168
Totals	54,225	4,181,139
Totals for Nebraska	111,151	8,117,775

<sup>\*</sup>These are the counties in the Sand Hill and High Plains regions where this dry rot is most prevalent.



Map showing the location of the Sand Hill and High Plains regions and indicating by name the counties each of which during 1909 produced 100,000 or more bushels of potatoes.

The losses from this dry rot have in some cases been very severe. The situation became so serious that during the spring

of 1909 the Alliance Commercial Club sent the potato growers of that region a circular letter warning them against the danger of planting infected seed. This warning was based upon the fact that during the previous fall some of the largest potato buyers had refused to handle these potatoes on account of the serious losses during storage due to this dry rot. The department of Agricultural Botany thru circular letters addressed to many of the buyers and commission men handling potatoes found that during certain winters their losses from rotting of the tubers had been as high as 20 to 60 per cent.

One of the most serious losses due to dry rot is the fact that the disease forces the immediate sale of the potatoes as soon as dug. This tends to demoralize the market and places the grower at the mercy of the buyers since he is himself afraid to store his crop and wait for better prices. Taken all in all this dry rot is perhaps the most serious potato trouble our farmers have to contend with in the Sand Hill and High Plains regions.

#### SYMPTOMS.

The dry rot here described is a strict tuber rot affecting mature tubers only. Neither the stems nor the young tubers are ordinarily in the least affected. Natural infection is known to occur solely thru wounds produced in the process of digging or subsequent handling. In many cases this rot secures a foothold thru wounds made by scab-producing animals of certain sorts and perhaps even thru scab spots due to fungus parasitism, tho the latter method is certainly very rare if we may judge from the laboratory experiments. The fungus cannot invade the tuber either about the "eyes" or thru the normal lenticels.

The rotting is rather slow and in general within four to six weeks from one-third to three-fourths of the tuber is destroyed. The epidermis of the rotted portion becomes slightly wrinkled and usually has a characteristic bluish color. On account of the rapid destruction of the underlying tissues the surface over these areas soon becomes distinctly depressed (Plate I).

The rot may make its appearance at any point on the surface of the tuber tho more commonly perhaps at the bud end of the tuber. There is no watery degeneration of the tuber unless other organisms gain entrance, so that this is in fact a dry rot.

#### MORPHOLOGY OF THE CAUSAL ORGANISM.

Previous to 1910 no comprehensive work on the genus Fusarium had appeared which could be considered authoritative and the genus was in a very chaotic condition. A species was defined by a few lines of description in which the name of the host played an important part. In 1910 appeared a publication entitled "Grundlagen einer Monographie der Gattung Fusarium (Link)," by Appel and Wollenweber. In this the authors have laid the foundation for a systematic treatment of the genus upon morphological characters.

During the fall of 1911 the morphological study of this organism, previously shown to be responsible for the dry rot of the Irish potato tuber herein described, was undertaken along the

lines laid down by Appel and Wollenweber.\*

The genus Fusarium was defined by Link in 1809, together with the allied genera, Fusidium, Fusisporium, and Atractium. From time to time Link dropped one or the other or combined them in various ways. In one of his late works in 1824 he defines the genera Fusisporium, Fusidium, and Fusarium, using the presence or absence of a thallus as prime character. As it happened the genera were thereby also divided on the basis of septation of spores, Fusisporium having non-septate spores.

Fusidium and Fusarium septate spores.

In 1824 Schlechtendahl introduced the use of the curvature of the spores as a character, which was also used by Corda in 1829. The latter distinguished Fusarium as having only curved spores and Fusidium as having both curved and straight spores. Later, 1837, he changed his views somewhat, giving Fusidium only straight spores, and Fusarium curved and straight spores, and dropped all forms which had pluri-septate conidia from these genera and used them to establish the genera Fusoma and Selenosporium, putting them under his Phragmidiaccae, while he puts his Fusidium and Fusarium under the Caeomaceae, and Fusisporium under the Sporotrichaceae.

Fries in 1845 reduced all these forms to two genera, Fusarium (Link) and Fusisporium (Link), but grouped certain organisms producing sickle-shaped spores in slimy layers into one genus

which be called *Pionnotes*.

Saccardo in 1886 divided the genus Fusarium into the following subgenera, Eu-Fusarium, Fusamen, and Leptosporium Eu-Fusarium was described as having cylindrical, spindle and sickle-shaped conidia with one or more septa, and was subdivided into Selenosporium (Corda) and Fusisporium (Link). Fusamen has similar conidia which are not septate, however, and Leptosporium has shorter, ovate or somewhat elongated non-septate conidia. Fusamen was divided into Selenospora and Fusispora.

<sup>\*</sup>An account of the morphology of the causal organism was submitted as part of a thesis for the M. A. degree from the University of Nebraska in May, 1912, by G. K. K. Link.

Saccardo also defines Fusidium, Fusoma, and Pinnotes; the former have spindle-shaped straight spores and the latter differs

from Fusarium in the nature of its spore layers.

Saccardo as well as Lindau and Appel and Wollenweber point out the great disorder and confusion which reign in this realm of mycology. Not only are the species of Fusarium not clearly defined, as they are mostly based on host descriptions, but the genus itself is not delineated. In their monograph Appel and Wollenweber 1910 have established the boundaries of the genus using Atractium (Link), Fusidium (Link), Fusisporium (Link), Sclenosporium (Corda), Fusoma (Corda), and Pionnotes (Fries) either in toto, or in part, as synonyms.\*

\* They define (l. c. page 61) the genus as follows:

"Konidien mehr oder weniger polar, meist dorsiventral, selten ausgesprochen radiär, mehr oder weniger gekrümmt, in der Reife gewöhnlich septiert, in Massen wenig oder lebhaft gefärbt, mehrere nacheinander an derselben Stelle erzeugt, aber nicht kettenartig verklebt, am Ende einfacher oder verzweigter, septierter Konidienträger, die entweder zerstreut zwischen den Hyphen, gelegentlich zu Coremien vereinigt, oder in Sporodochien gesellig auftreten. Konidien später pulverig zwischen den Hyphen zerstreut oder als tuberculariaähnliches, fest begrenztes, gallertiges Sporodochiumpolster, gelegentlich, als *Pionnotes*. in unbegrenzten schleimigen Lagern auftretend.

"Chlamydosporen, oval oder birnenförmig, einzeln oder gesellig, in Ketten oder Knäueln, dauernd zusammenhängend, terminal oder intercalar, nicht mehrere nacheinander erzeugt, ohne besondere von den Konidienträgern zu unterscheidende Träger, auch in Farbe kaum besonders hervortretend. Nie in gallertigen Lagern dichter zusammen-

geschart.

"Hyphen sentiert, verschieden verzweigt, eni- und endophytisch, spärlich oder reichlich auftretend, entweder isoliert oder zusammen ein lockeres oder dichteres, teilweise zu coremienartigen oder, besonders als Stroma, zu plectenchymatischen gestaltlichen oder gestaltlosen Verwachsungen, ferner häufig auch zu immersem Wachstum neigendes, begrenztes oder ausgebreitetes, vielfach durch Anastomosen innig zusammenge-

schlossenes, gelegentlich lebhaft gefärbtes Mycel bildend.

"Bemerkung. Es ist unentschieden gelassen, ob Arten ohne Konidiensentierung aus der Gattung ausscheiden, dagegen nicht, wenn sie wenigstens eine Neigung zur Sentierung haben (F. orthoceras), oder ob erstere sich mit Saccardo zu einer Untergattung *Fusamen* vereinigen lassen; ferner, in welcher Folge die Merkmale für die Bestimmung einer Art als Fusarium entscheidend sind, wobei die Wahl ist zwischen: tierung, Dorsiventralität, Polarität, Längsachsenkrümmung der Konidien. Sehr zweifelhaft erscheint eine Beziehung von Fusarium obiger Auffassung mit den von Saccardo unter Leptosporium vereinigtene Arten, doch kann nur die Untersuchung vieler Formen diese Frage der Begrenzung des Gattungsbegriffs klären. Was de Farbe der Konidienmassen anbelangt, so scheinen schwarze Farben nicht normal aufzutreten, auch beim Mycel nicht. Helle, Orange und Ockerfarben herrschen bei Konidien vor, für das Mycel treten noch gelb, rot, blau hinzu. Der Begriff Sklerotium ist für Fusarium diskutierbar. Die Untersuchungen haben nichts ergeben, was die Sonderstellung irgend welcher plectenchymatischer Gebilde als Sklerotien notwendig erscheinen liesse."

Appel and Wollenweber have worked over a great number of Fusarium species and made it their first object to determine which characters in this genus can be used as general, reliable species characters. They also came to definite conclusions concerning the nature of the medium and the cultural conditions which will give these characters most uniformly in their true form and have designated these as "normal" because they offer each Fusarium the conditions "Bei der er (der Pilz) im Stande ist, seinen Entwickelungsgang normal abzuschlieszen." They make use of the following characters: form of conidia; presence or absence of chlamydospores; color of the conidia; septation; mycelial color; width of the conidia; and absence or presence of plectenchyma-like stroma masses.

They found that boiled potato tubers and boiled potato stems furnished a normal substratum when kept at a temperature

ranging from 12° to 25° C., and in diffuse light.

Cultures grown on gelatin and agar media are not normal and cannot be used in the determination of characters. For the bacteriologist these media have arbitrarily been made normal, but for the mycologist who works with higher fungi they are only of secondary use as Brefeld 1905 points out and as is well borne out by the great masses of conflicting data which have accumulated around many of our species. It is well known that agar colonies often show characters which the fungus ordinarily does not show and that more often not all of the characters of the fungus are realized on these media.

The usual host tissue should be used as much as possible as a substratum, and descriptions should only be made from such cultures. In fact, there is no reason why the mycologist, who works with a vastly more complex mixture than many a chemist does, should not adopt the exact methods of description which the chemist uses. The bacteriologist already has done this. Thus, the normal culture medium for each genus should be determined: and then the nature and kind of the medium and the conditions of light, temperature, and moisture should be clearly stated with each new description of the fungus, so that any one can repeat the work and that all work will be done under the same condi-This will involve a great deal of labor, but the conditions which will enable us to make determinations of reliable characters of certain complicated genera will have to be determined before much headway can be made. In these studies the characters used by Appel and Wollenweber were employed and the results show that they are reliable characters under the conditions of the experiment. It has also been found that all of these characters can be modified from the normal by variation in the cultural conditions.

Appel and Wollenweber's monograph carries a full bibliography and no attempt is made to duplicate it here. It is evident that if our knowledge of the taxonomy of the genus has been in chaos prior to 1910, the knowledge of the pathological conditions which these organisms bring about has been in no better condition, because the work done cannot be linked to any definite organism and because no reliable isolation and inoculation experiments have been made in many cases.

#### OCCURRENCE OF THE ORGANISM.

The organism has repeatedly been isolated by the writers from potato tubers affected with dry rot. If the rot has progressed only slightly, a sunken, wrinkled spot appears on the surface of the tuber and usually no exterior signs of the fungus are visible. As the rot progresses the fungus area increases and finally the potato presents a shrunken, wrinkled appearance. Later in the progress of the disease small tufts of hyphae, which are pink in color, may appear on the surface but these should not be called sporodochia (Pl. I).

If one cuts into a partly rotted tuber, a part of the tuber will be found perfectly sound and the other part, which borders on the spot, made up of disintegrated potato tissue, of hyphae and conidia. The potato tissue has a brownish color and is mealy and dry. Here and there large cavities appear, due to rents, which are formed as the cells shrink away from each other, and which are often filled with hyphal masses of a whitish color (Pl. X, fig. 1). When such a potato is cut open and exposed to light

the fungus soon takes on a pink color.

If potatoes infected with this organism are kept in rather moist conditions, or if the outer crust is not ruptured, the fungus may eat out the entire contents of the tuber, leaving merely the cork layer (Pl. X, fig. 1). Often pure cultures can be obtained at first trial from such a tuber, because of the fact that the fungus leaves behind itself a crust of hard, dry starch as it makes inroads on the tissue, thus shutting out other fungi. If the wound is open, secondary infections occur, and often bacteria and other fungi, such as Penicillium and Verticillium, are found with the Fusarium. Frequently a wet rot precedes the dry rot if the proper causal bacteria get in (Pl. II). If an inoculation is made by cutting the surface of a tuber and placing the inoculum on it, thus letting the fungus work down and form a crust as it goes, secondary infection rarely sets in. If, however, an inoculation is made by puncturing the skin, thus leaving a hole, bacteria generally get in.

#### TECHNIQUE.

In their studies Appel and Wollenweber tested all sorts of media and came to the conclusion that the normal conditions for Fusarium culture could be realized on sterilized vegetable substrata. They used as much as possible the tissues of the host

on which the fungus is found.

In the course of these studies with Fusarium tuberivorum, sterilized potato plugs, sterilized potato stems, raw potato plates, glucose agar, beef bouillon (+10), sawdust soaked with potato-vine extract, filter paper soaked with a synthetic solution of the same composition as the glucose agar, and potato agar were used as solid culture media. Various liquid media, such as glucose solution and water, were used.

The plugs were prepared from potato tubers grown under irrigation and put into Roux tubes whose container was half filled with water. The tubes were then plugged and sterilized by steaming on three consecutive days at 100° C. The first day they were steamed for a period of one-half hour and on the other days for a period of 15 minutes. Pieces of young potato vines were prepared in the same manner.

The glucose agar used was of the same composition as that used by Appel and Wollenweber with the exception that 15 grams

of agar were used instead of 10 grams.

The following is the formula for the agar:

1,000 c.c. water.

20 grams ammonium nitrate.

10 grams di-potassium phosphate.

5 grams magnesium sulfate.

100 grams glucose.

15 grams agar (Witte's powdered).

The chemicals were Merck's and chemically pure. The agar was dissolved in the water, autoclaved and then the other ingredients were added and the solution sterilized on three consecutive days at 100° C, in streaming steam.

Cultures were also grown in hanging drops of agar prepared in the above manner and in distilled water. By using these drops we were enabled to study the progress of a single spore's development for nine days. Some cultures were also grown in Erlenmeyer flasks in distilled water, 1 per cent glucose, and in a solu-

tion of the following composition:

1,000 c.c. water.

20 grams ammonium nitrate.

10 grams di-potassium phosphate.

5 grams magnesium sulfate.

100 grams glucose.

A few cultures were grown on filter paper soaked with a 1 per cent glucose solution. A potato agar of the following composition was used:

500 c.c. water.

100 grams glucose.

500 grams potato extract.

15 grams agar.

Potato plugs, raw potato plates, and stems were used for the determination of the morphological characters; while the agar was used for the isolation, for color study, and for the study of the rapidity of growth of the organism. The liquid media were used for color study and for spore study.

The growth of the fungus was studied at the following temperatures and humidities (the humidities given being those of the

air which surrounded the tubes or plates in question):

8° to 10° C., (humidity 60 per cent); 25° to 27° C., (humidity 98 per cent); 20° to 25° C., (humidity 40 to 55 per cent); 22° to 35° C., (humidity 40 to 55 per cent); 25° to 45° C., (humidity 10 to 40 per cent); 1.1° C., —3.9° C.; and —22° C.

Cultures were grown in direct sunlight, in diffuse light, in darkness, and under double walled bell jars filled with solutions of copper sulfate and ammonium hydrate, and potassium bichromate prepared according to Bulletin 55 of the Bureau of Plant Industry, page 49. Cultures in the dark were made by wrapping the Roux tubes with the black paper which is used in wrapping

photographic plates.

The original isolation was begun by opening an infected tuber and cutting out pieces of the tissue near the affected area with sterilized needles. These pieces were then transferred to agar plates and no contaminations of any sort appeared. When the spore material was examined it was apparent either that there were six to eight types of Fusarium in the culture or that it was a pure culture showing a great diversity as to spore form. Consequently it was absolutely necessary to isolate single spores and then grow these to determine whether these spore forms were all from one progenitor or from several.

Various methods were employed by the writers for single spore isolation; but none, save one by which they could actually examine the inoculum before inoculation and determine whether a single spore was present or not, is satisfactory and reliable. The use of the ordinary dilution and pouring method is admissible after one has pure cultures and when the spores do not stick together. The same thing applies to the streak plate method. The examination of poured plates is a cumbersome and in-

adequate method if one has to determine positively whether one is dealing with single spores or not. The writers found that the following method is quite easy, absolutely safe, and rigidly scientific: A small amount of culture material is put into about 1 c.c. of sterilized water and then well shaken and stirred. The suspension is then poured into a petri dish, and a few drops about the size of a pin point, which are removed with a platinum wire with a minute loop, are examined and the spore content determined. If so dilute that one gets one or no spores, single drops are then put upon sterile cover-glasses and examined on a sterile hanging drop slide with the microscope. If a spore is present, the cover-glass is transferred to an agar plate by shoving it into the agar medium, drop side up, until the drop touches the surface of the medium. A little sterilized water is then poured on the agar, and the plate covered and rolled so as to distribute the suspension. If this work is done in an inoculation cage\* and if one works cleanly and carefully, it can be done without danger of contamination. If contaminations do set in, transfers are made as soon as the colonies appear, and in this manner absolute single spore cultures are gotten. Plantings made in this manner when more than one spore was counted in the drop show the required number of colonies. The contaminations which ordinarily appear are Mucor, Penicillium, and Aspergillus.

If one allows the spores to germinate in the petri dish before the actual mounts are made, detection of the spores under the lens is facilitated, and in this manner the smallest of spores can be found. Sooner or later all who expect to have *pure cultures* which are really pure cultures will have to follow the advice of

Brefeld (14:87).†

The color determinations were made according to the Repertoire de Couleurs published by the Société Française des Chrysanthemists 1905.

#### MACROSCOPIC CHARACTERS.

Studies for the determination of the gross aspect of the fungus were made with cultures on glucose agar and on beef bouillon.

<sup>\*</sup>Wilcox, E. M. and Link, G. K. K. 1912. A new form of pure culture chamber. Phytopathology 2:120. Fig. 1.

<sup>† &</sup>quot;Für die Durchführung zuverlässiger mycologischer Untersuchungen ist die Gewinnung des Sporenmaterials von den verschiedenen Pilzen in reiner Form das erste und unerlässlichste Erfordernis. Von dem reinen Sporenmaterial kann nur durch die Aussaat der einzelnen Spore und ihre continuierliche Verfolgung in allen Stadien der Entwicklung in den geeigneten, durchsichtigen pilzfreien Nährmedien ein sicheres Resultat gewonnen werden, welches in den einzelnen Fällen der weiteren Ergänzung bedarf durch die Aussaat rein gewonnenen Sporenmaterials in sichersterilisierten und zusagenden, nach aussen geschützten Massensubstraten."

In these the growth of the fungus at first is appressed, appears white in color, and the edge of the colony is marked by a fluffy, white, aerial mycelium. This fluffy edge is characteristic of cultures thruout their entire development. The central part of the colony produces conidia first on aerial hyphae, which soon collapse and allow the conidia to fall on the hyphal mat and give it a pink color. If secondary germination of conidia sets in, the whole mass is covered by another mat of aerial hyphae, which often do not go over to conidia formation, and consequently the culture looks more white than it normally does. When the culture has been under favorable conditions of light and moisture. an enormous number of conidia are formed, which fall down to the substratum, so that we get concentric rings of heaps of hyphae and conidia, which look like series of embankments (Pl. XXII). The whole colony appears powdery after two weeks' growth. the agar a very firm plectenchyma is formed, which sets up tensions, pulling the mass into wrinkles as it grows older. stroma-like or sclerotial bodies are formed.

On some potato tubes masses of mycelium which were black or dark blue in color were formed if the oxygen supply was cut down. According to Appel and Wollenweber the appearance of sclerotia need not be considered as a taxonomic character. They are by far not as pronounced in Fusarium tuberivorum as they are in Fusarium orthoceras according to Appel and Wollen-

weber's and the writers' observations.

#### THE MYCELIUM.

In this organism we have to consider a complex of hyphal threads. Under proper conditions the hyphae fragment terminally into colonies of cells, usually called spores or conidia, which may be made up of from one to eight cells. That we have colonies is indicated by the fact that these spores break up into oidia when put into unfavorable conditions such as are realized when the fungus is grown on beef bouillon or in distilled water. On beef extract the mycelium itself can be made to fragment intercalarally into such colonies. Such fragments, however, do not show the end differentiation which the end cells of the usual colony At times, in tube cultures, and in distilled water, the mycelium fragments terminally or intercalarally into round, thick walled, simple, or compound colonies which usually are smooth and full of oil. On raw potato plates such colonies with spiny walls were noted and these can be called chlamydospores.

Under other conditions the mycelium vegetates as such without spore formation, especially if the temperature is low and if there is an abundance of food. The same condition was realized

in cultures in 1 per cent glucose solution and in distilled water. In the former case there probably is so much food that the mycelium can maintain its growth without going over to spore formation, while in the latter the growth is so slow and impoverished that septation cannot set in.

Spores are borne on conidiophores of varying complexity. Generally the conidiophores are arranged on one side of a hypha and are simple, unbranched, one to several celled, sterigma-like structures. A compound tristerigmate conidiophore has been found, and all gradations between this and the simplest have

been obtained by varying the cultural condition.

The fungus on its natural host makes a loose growth and takes the form of an aerial mycelium when it has access to cavities. Before the cavities are entirely filled, and in moist tube cultures, the peculiar arrangement of the hyphae which puts this fungus under the Tuberculariaceae is very apparent.

No sporodochia-like structures are found on agar at all. times on agar, especially if a little normal lactic acid has been added to it, a slimy *Pionnotes* appearance is realized. This has

also been found off and on on glucose agar.

#### SPORE SEPTATION.

Counts of spores and measurements were made from cultures all of which had been started from the same spore and grown under various environmental conditions. All of the detailed study was made with the progeny of a single, three-septate, curved spore. This course was adopted only after the writers had satisfied themselves from studies of colonies which had come from 50 single spores that they all behaved alike. It makes no difference whether a single celled spore is used or a three-septate spore, the results are the same. A three-septate curved spore was used so that the variations which do appear might be as apparent as possible and because there can be no doubt that such a spore is a true Fusarium spore.

These studies were made with cultures which were grown on potato plugs in an incubator, temperature 25° to 27° C., (humidity 98 per cent), wrapped in black paper, in a refrigerator, temperature 8° to 10° C., (humidity 60 per cent), wrapped in black paper, in the room in diffuse light, and in the room, wrapped in black paper, temperature 20° to 25° C., (humidity 40 to 60 per cent). From these cultures counts were made every week for four weeks to determine the fluctuation of the number of septations per spore. These results are plotted in curves, Tables 2 to 5. Graphs 1. 2, 3, and 4. Besides these tabulated counts, several hundred were made from cultures on potato plugs, agar, and raw potato, and

all show a great preponderance in the number of the one-septate spores. This number ranges from 40 to 98 per cent according to the age of the culture, the one-septate spores increasing in number while the two- and three-septate spores decrease in number with

the aging of the colony.

The results show that for a definite medium under definite conditions the percentages of the number of septations are a reliable character. Appel and Wollenweber distinguish an "An," "Norm," and "Ab-Kultur" and a "Jung," "Hoch," and "Alt-Kultur." The conidia which are produced from the mycelium of an original isolation inoculation would be "An-Kultur" conidia. These are unfit for character studies. When such conidia of the "An-Kultur" are used as inoculum, the resulting culture is a "Norm-Kultur," which can be maintained for a long time by repeated transfers. If degeneration should set in, the "Ab-Kultur" condition would result. They divide the "Norm-Kultur" into "Jung," "Hoch," and "Alt-Kultur." In the former the conidia may be abnormally large or small and irregular in form, consequently not suitable for measurements and form study. In the "Hoch-Kultur," which sets in after a period of eight to fourteen days, the greatest uniformity is noticed and this condition may last for a month. Poorly developed forms which develop late in the "Norm-Kultur" constitute the "Alt-Kultur."

No striking differences in the appearance of the conidia were found in the various cultural stages of Fusarium tuberirorum excepting poorly developed conidia which can be placed in the "Alt-Kultur." All of the work was done with the organism in the "Hoch-Kultur" condition so that there might be uniformity in the data. In the "Alt-Kultur" the spores dry up, curve considerably, and often one end cell becomes very pointed and hyalin. When this condition is reached the percentage of one septate conidia is very high, 85 to 95 per cent. At times cells of such a spore seem to have varying amounts of plasma, one remaining plump and round and the other drying up and be-

coming pointed.

The percentages of numbers of septation per spore are surprisingly constant, but can be influenced by changes in the environmental conditions. Especially is this the case when the temperatures are varied. (Tables 2 to 5, Graphs 1 to 4.)

Counts have repeatedly been made of the number of spore septations of Fusarium orthoceras, of Fusarium tuberivorum, and of what may be another Fusarium which attacks Colorado potatoes, and we find that for these three species the counts of the number of septation of the spores would be sufficient to separate them. When in addition one makes use of other char-

acters there can be no reason why the characters decided upon by Appel and Wollenweber should not give a good working basis

for the genus.

In this fungus not much difference can be noticed in the rapidity of the growth of the cultures, whether one begins with conidia or with mycelium as inoculum. It was noted that a 15-months-old culture on rice which was as dry as powder gave viable conidia, which had not at all lost their pathogenesis for tubers.

The spore walls are quite thick. Single-celled or two-celled colonies are by far more resistant to collapse of the wall than those of a higher number of cells; in fact it seems probable that the walls which set in in a growing cell are a response to a pressure stimulus. The diameter of septa suffers practically no shrinkage even under the driest condition and consequently gives a very constant and reliable basis for measurement. When the cell wall of a spore collapses, the spore looks very much like a series of hourglasses (Pl. XXVII, figs. 14-15, 20, and 22).

When the fungus is grown on agar, the cells swell greatly while the septa do not, and then we get a spore which looks very much like an inflated rubber tube constricted by bands at regular intervals. Sometimes these appear in colonies on agar and are then associated with a slimy appearance so that one could easily mistake them for a new species or for a subspecies. In fact, both the dried-out form and this swollen form have off and on

been described as new species.

If the spores figured by Appel and Wollenweber 1910, page 38, as Fusarium didymum were bent slightly on the cross wall as an axis, and if one end were bent a trifle more than the other, we would have a spore such as presented by Fusarium tuberirorum. In the one-septate spores one end usually is a little more plump than the other. Some of these spores, however, show a true sickle shape. By far the most spores in the pure cultures, even as many as 98 per cent, are one-septate. At times the basal ends show remnants of the so-called foot, but it is a very difficult matter to decide which end is basal or apical after the spore is once off the sterigma.

Some of the higher septate spores show the shape of Fusarium coeruleum, others that of Fusarium sp. (Appel and Wollenweber 1910, fig. S, page 38). A combination of Fusarium coeruleum and of Fusarium solani would in many cases give us the shape of the typical spore of Fusarium tuberivorum. Often the nonseptate spores are clavate. This condition is very frequently found on beef bouillon or in cultures which have been grown in distilled water (Pl. XXVII, fig. 13). When colonies or spores are put into water all of the spores are of the clavate shape.

There is a great range in the form of these spores. A spore may have an almost straight ventral axis and a dorsal axis with a curvature like that of *Fusarium* sp. (See figure 8, page 38 of Appel and Wollenweber 1910 and Pl. XXIV, figs. 30, 33, 34, 45, 51, 57, and 60 of the present bulletin), or it may have a ventral axis which is straight up to the last septum at the apex and then bends in the same manner as does *Fusarium solani* (Pl. XXIV, fig. 4), and a dorsal axis which is decidedly more humped at the two apical septa than is that of *Fusarium solani* (Pl. XXIV, fig. 40; Pl. XXIII, figs. 3, 4, and 9).

That the spores can develop a specialized apical and basal cell is shown by cultures which were made on potato plugs grown at the temperatures 8° to 10° C. Growth was very slow under these conditions and the percentage of three-septate spores, which ordinarily does not run over 10 per cent, went up to 40 per cent. The spores remained attached to the conidiophore for a long time, developed slowly, and showed all stages in the development of the peculiar structures which we find on the

apical and basal cells of a spore.

The apical cells show a tapering such as Fusarium discolor and Fusarium rubiginosum do, excepting that the end is a little more blunt than in the former (Pl. XXIII, fig. 18). The basal cells show all gradations in the development of the foot or "Stiefel." Practically all the types are found here. Hanging drop cultures show that the elongation of the apical cell is determined before it gets to be a conidium, i. e., when the basal cell of the preceding conidium is developed. There is a pulling of each end much like the pulling of wax, causing the basal end of the conidium to taper in the opposite direction. In rapidlydeveloping conidia the cell rounds off quickly and is cut off so quickly that these peculiar end structures are not developed. This is a predominating condition in the spores of Fusarium tuberivorum. When the medium becomes exhausted or when a fungus develops slowly and has an opportunity to mature before it germinates, the end cells are developed.

The origin of the little papillae-like structures which are apparent as a heel of the boot has been nicely indicated. Appel and Wollenweber 1910 in fig. S, 2, page 38, figured a spore with two such papillae. Fusarium didymum shows traces of these, and all the others show various stages of degeneration of these papillae. When the organism is grown in beef bouillon, fragmentation of these colonies sets in so slowly that the origin of these swellings becomes apparent. They are remnants of the outer walls which are left as two adjoining spores separate by their cross walls. Cells separate last in the middle of the septa,

thus producing a pulled out appearance (Pl. XXV, figs. 2, 4, 5, and 7). This last point of contact gives rise to the toe, one edge formed by the wall to the foot, and the other edge is usually lost (Pl. XXV, figs. 2, 4, 5, and 7), but retained in such spores as we find in figures S and L on page 38 of Appel and Wollenweber 1910. Slow growth favors the development of these parts, while in rapid development they are lost. The one- and nonseptate spores which are developed rapidly show no such modification. In general we do not find that the structure of the basal and apical cell of this species develops anything that can be considered sufficiently characteristic to be used as a specific character. All sorts of stages are shown in the plate, so that each one can judge for himself, as it is possible that one who has studied a great number of species of Fusarium will be able to pick out the characteristic form which can be set aside for this particular species.

The following are some counts of spores from a culture:

On glucose agar, 115 days old, dry.

0-septate										٠	1	per	cent.
1-septate	٠	٠		٠						٠	98	per	cent.
2-septate	٠		۰						٠		1	per	cent.
3+-septate.							d				Ve	ry :	few.

On potato plugs in Roux tubes, some water left in the bulb. 130 days old, ochre-color.

0-septate.	٠	,	٠		٠	۰						٠	.Few.
1-septate.				٠									.98 per cent.
2-septate.													
													.Very few.

In refrigerator, potato plugs moist, temperature  $8^\circ$  to  $10^\circ$  C., humidity 60 per cent, 70 days old.

	P.		
0-septate.	 	20 per c	ent.
1-septate.	 	55 per c	ent.
2-septate.	 	10 per c	ent.
		13 per c	
4-septate.	 	3 per c	ent.
0-septate.	 	18 per c	ent.
1-septate.	 	71 per c	ent.
2-septate.	 	10 per c	ent.
3-septate.	 	1 per c	ent.
4-septate.	 	Few.	

The average of six tubes which were partly dry and partly moist was:

0-septate.												
1-septate.												
2-septate.												
3-septate.												cent.
4+-septat	e.									.Fe	w.	

In incubator, potato plugs moist, temperature 25° to 27°C... humidity 98 per cent, 70 days old.

0-septate.														7	per	cent
1-septate.			٠				٠	,			e			82	per	cent.
2-septate.		٠				٠			۰					8	per	cent
3-septate.													i	2	per	cent.
1sentat	0													Tra	157	

### Potato plug dry.

0-septate	 4		٠	٠								. 4	2 per	cent.
1-septate.											٠	.73	l per	cent.
2-septate.	,											.20	) per	cent.
3-septate.					6			۰	٠			. }	5 per	cent.
1+-septate													-	

#### Average of six tubes.

0-septate.		٠			٠						. 4	per	cent.
1-septate.													
2-septate.													
3-septate.						٠			,		. 4	per	cent.

Tubes wraped in black paper, potato plugs dry, temperature 22° to 25° C., humidity 40 to 55 per cent, 70 days old in room.

U-septate				٠	٠	٠	٠	۰	,			. Few.
1-septate							٠					.98 per cent.
2-septate	۰			,								. 2 per cent.
3+-septate						۰						. Few.

### Potato plug wet.

0-septate.																
1-septate.				٠	٠		٠	۰				٠		77	per	cent.
2-septate.						٠	۰			۰				13	per	cent.
3-septate.						۰			4	٠				5	per	cent.
4+-septar	te.										٠	۰		Fe	W.	

### Potato plug always dry.

3	6-										
0-septate.											
1-septate.										٠	.98 per cent.
2-septate.											
3-septate.	•						٠		٠		. Few.

20 1005000000 100. 1
Grown in distilled water, temperature 25° to 27° C., 8 weeks old, in light and in dark; few spores.  0-septate
Grown in 1 per cent glucose, immersed, 4 weeks old, no conidia.
From raw potato, culture dry, 150 days old, ochre-color.
0-septate       23 per cent.         1-septate       69 per cent.         2-septate       7 per cent.         3+-septate       1 per cent.
0-septate       30 per cent         1-septate       67 per cent         2-septate       2 per cent         3+-septate       Few
Averages from a great number of potatoes taken at random, counted at different times, gave the following results:
0-septate
Grown on glucose agar, 4 days old.
0-septate       20 per cent.         1-septate       70 per cent.         2-septate       8 per cent.         3-septate       2 per cent.         4+-septate       Few.
Same culture 52 days old.
0-septate       7 per cent         1-septate       84 per cent         2-septate       10 per cent         3+-septate       Few
On stems 7 days old, in light.
0-septate       .16 per cent.         1-septate       .49 per cent.         2-septate       .23 per cent.         3-septate       .11 per cent.
On stems 48 days old, dry, in light.
0-septate

Table 2.—Representing in percentages the fluctuation of spore septation of a culture grown in a refrigerator, temperature 8° to 10° C., humidity 60 per cent.

-		February 16	February 23	March 1	March 8
0-septate.	$\begin{array}{cccc} \text{Tube A} & \dots & \\ \text{B} & \dots & \\ \text{C} & \dots & \\ \text{D} & \dots & \end{array}$	7 0	17. 8 3	15 10 3 9	32 12 7 17
	Average	2	8	9	17
1-septate.	$\begin{array}{ccc} \text{Tube A} & \dots & \\ \text{B} & \dots & \\ \text{C} & \dots & \\ \text{D} & \dots & \end{array}$	30	51 40 47 46	57 43 43 60	56 53 61 74
	Average	27	46	51	61
2-septate.	$\begin{array}{ccc} \text{Tube A} & \dots & \\ \text{B} & \dots & \\ \text{C} & \dots & \\ \text{D} & \dots & \\ \text{Average} & \dots & \end{array}$	28 20	19 21 18 19	$   \begin{array}{c}     16 \\     21 \\     15 \\     \hline     16 \\     \hline     17   \end{array} $	7 18 20 6 13
3-septate.	Tube A	44 36 42 54 44	12 31 32 32 27	12 26 39 15 	5 17 12 3 9
4-5-septate.	Tube D	7			

Table 3.—Representing in percentages the fluctuation of spore septation of a culture grown in an incubator, temperature 25° to 27° C., humidity 98 per cent.

		February 16	February 23	March 1	March 8
0-septate.	Tube A	35 5 12 17	5 6 7 15	8 16 6 23	5 26 41 15
	Average	17	8	13	22
1-septate.	Tube A B C D	57 77 67 77	85 90 80 76	83 66 84 75	80 72 56 85
	Average	67	83	77	73
2-septate.	Tube A B C D	8 16 9 8	6 4 9 5		12 2 3 0
	Average	10	6	7	4
3-septate.	Tube A	0 2 12 5	4 0 4 4	1 5 4 1	3 0 0 0
	Average	5	3	3	1

Table 4.—Representing in percentages the fluctuation of spore septation of a culture grown in a room, temperature 20° to 27° C., humidity 40 to 55 per cent.

		February 1	6 February 23	March 1	March 8
0-septate.	Tube A	8 5 5 8	12 12 4	7 2 11 10	17 9
	Average	7	9	8	13
1-septate.	Tubé A B C D	68 72 73 88	79 76  82	88 83 77 82	78 81
	Average	75	79	83	80
2-septate.	$\begin{array}{ccc} \text{Tube} & \text{A.} & \dots \\ & \text{B.} & \dots \\ & \text{C.} & \dots \\ & \text{D.} & \dots \end{array}$	18 13 13 1	6 8 	3 10 10 7	5 7
	Average	11	9	8	6
3-septate.	Tube A B C D	6 10 9 3	3 4 	2 5 2 1	3
	Average	7	3	3	1

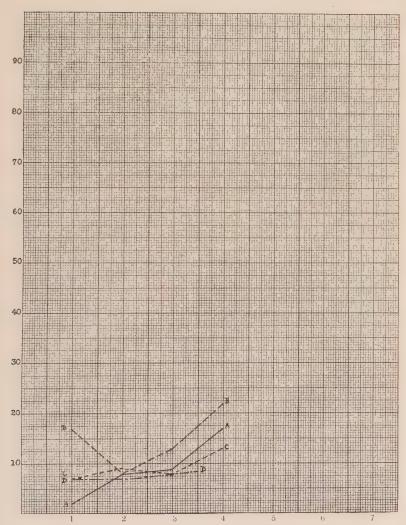
Table 5.—Representing in percentages the fluctuation of spore septation of a culture wrapped in black paper, grown in a room, temperature 20° to 27° C., humidity 40 to 55 per cent.

		February	16, Fe	ebruary 23	March 1
0-septate.	Tube A	5 4 12 7		7 8 8 6	6 9 10 6
	Average	7		7	. 8
1-septate.	Tube A B C D.	79 78 85 70		82 79 79 79	85 87 75 82
	Average	78		80	83
2-septate.	Tube A	-		7 11 6 11	8 2 9 10
3-septate.	Average  Tube A	10 2 9 1 10		9 4 2 7 4	8 1 1 6 2
	Average	6		4	3

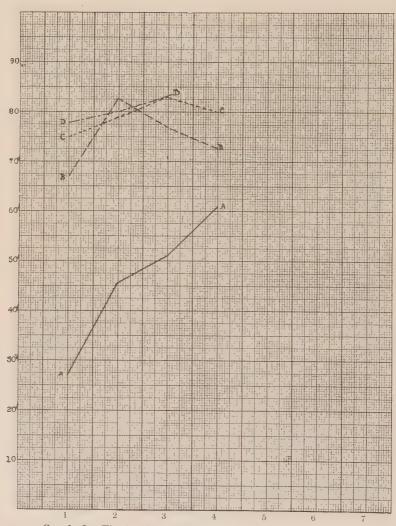
In these tables each figure represents the average of at least six counts. The cultures studied in this case were started with inoculum from a culture which had been started with a single, three-septate spore, five weeks old, and showed the following percentages of spore septation:

0-septate.									٠				6	per	cent.
1-septate.		٠		٠									83	per	cent.
2-septate.															
3-septate.			٠	۰	٠			٠			,		$^{2}$	per	cent.

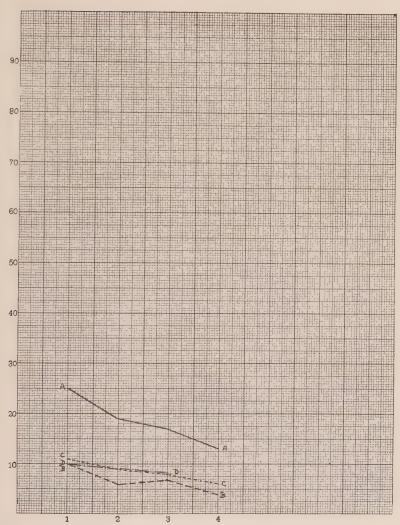
Graphs 1, 2, 3, and 4 are plots of the figures represented in Tables 2, 3, 4, and 5. The percentages are plotted on the ordinates and the number of weeks on the abscissac. In each graph, line A represents the cultures grown in the refrigerator, line B the cultures grown in the incubator, line C the cultures grown in the room, and line D the cultures wrapped in black paper and grown in the room.



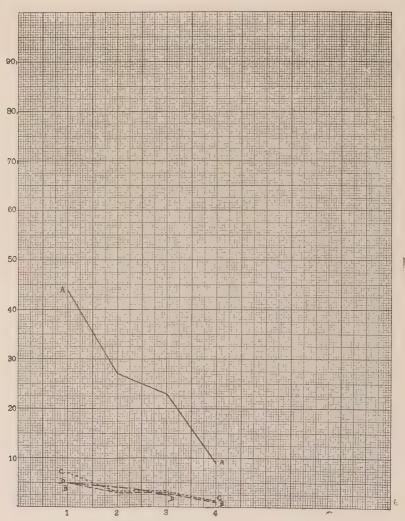
Graph 1. Fluctuation in the number of non-septate spores.



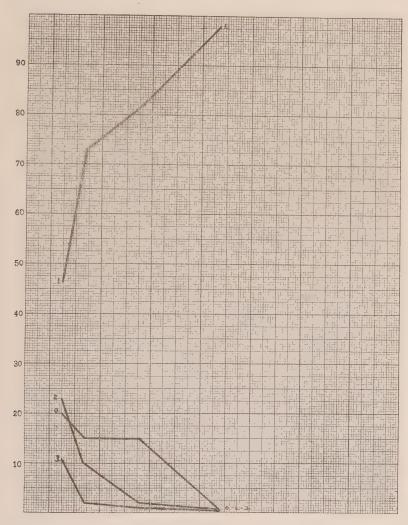
Graph 2. Fluctuation in the number of 1-septate spores.



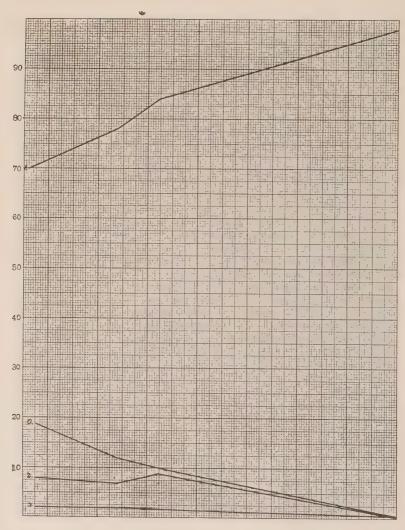
Graph 3. Fluctuation in the number of 2-septate spores.



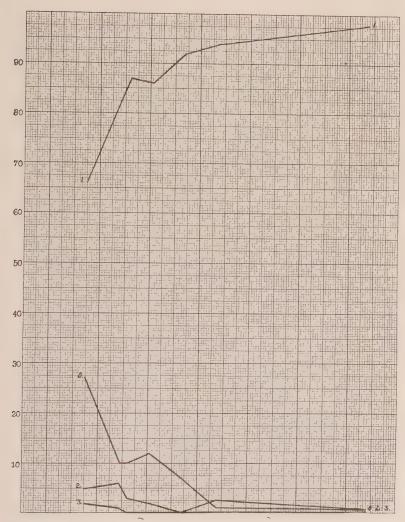
Graph 4. Fluctuation in the number of 3-septate spores.



Graph 5. Fluctuation in spore septation of culture grown on potato stems for seventy-eight (78) days.



Graph 6. Fluctuation in spore septation of culture grown on glucose agar for one hundred and fifty (150) days.



Graph 7. Fluctuation in spore septation of culture grown on potato plug for one hundred and thirty-eight (138) days.

Graph 1 represents the fluctuation in the number of non-septate spores, Graph 2 the fluctuation in the number of one-septate spores, Graph 3 the fluctuation in the number of two-septate spores, and Graph 4 the fluctuation in the number of three-septate spores. Graph 5 represents the fluctuation in the number of spore septation of a culture made from the same inoculum as cultures of Graphs 1, 2, 3, and 4 and grown on potato stems for 78 days. Counts were made when the culture was 15, 24, 47, and 78 days old. Graph 6 represents the fluctuation in the number of spore septation of a culture made from the same inoculum as cultures for Graphs 1, 2, 3, and 4, grown on glucose agar for 150 days. Counts were made when the culture was 5, 37, 55, and 150 days old. Graph 7 represents the fluctuation in the number of spore septation of a culture made from the same inoculum as the culture for Graph 6 and grown on a potato plug for 138 days. Counts were made when the culture was 25, 39, 65 and 130 days old.

In Graphs 5, 6, and 7 each space on the abscissa represents one day and every two spaces on the ordinate one per cent. Lines 0, 1, 2, and 3 represent the number of 0-, 1-, 2-, and 3-

septate spores respectively.

From these graphs it is apparent that the number of 1-septate spores increases with the age of the culture and that the number of 2- to 3-septate spores decreases. This holds for all sorts of media and substrata. It also is apparent that light has little influence on the percentages, at least not more than the error of the count, that moisture has some influence and that temperature, especially low temperature, has the most decided influence. Low temperatures retard growth, consequently a spore is slowly cut off, does not germinate into a hypa at once, but rather grows slowly and becomes septate. The multicellular spore is really only a slow or delayed form of germination of a single celled spore or true conidium. Observations of single-celled spores show that they are just as able to germinate as any other. The many-celled spores when put into water break up into their component parts which can germinate under proper conditions.

As the food supply becomes exhausted, and this is soon realized on the outside of the mat of a colony, the 2- to 3-septate condition drops off and the 1-septate condition runs up to 90 to 98 per cent. In tubers, when the fungus has sufficient food but where a sudden check is put on growth due to desiccation, the percentages of non-septate spores run high. The spores ordinarily fall off the sterigmata when in the non-septate condition; often, however, especially if growth is slow, several septa may appear before the spore falls off. Under favorable conditions a single-

celled spore is formed, the sterigma grows rapidly and shoves the spore off. If sufficient food is present, the spore germinates at once either into a hypha or into a several-septate spore. Under the most favorable conditions, however, the spore germinates at once into a hypha. When the food supply gives out, the one-septate condition is the most prevalent and, as mentioned above, many of these spores show only one viable end. At times the spores germinate directly into other conidia (Pl. XXVII, figs. 23 and 24). Very often the conidia fuse and then we have an appearance like that presented by the basidiospores of *Tilletia*, joined up and producing other conidia (Pl. XXVII, fig. 10). Fusions in the mycelium are often found.

## INFLUENCE OF TEMPERATURE.

As mentioned above, temperature has a decided influence on the septation of the mycelium. The fungus grows best at temperatures ranging from 22° to 27° C. Temperatures above this retard the growth, and when the organism is subjected to temperatures ranging from 40° to 55° C., no growth sets in. At 1° C., -22° C., and -3.9° C. no growth takes place, but when the fungus which was kept at 1° C. for a period of three weeks is brought into a temperature of 3.3° C., growth begins. and when the cultures were kept at -22° C. and -3.9° C. for two weeks and then gradually brought up to the temperature of 3.3° C., no growth was noted. When these were put into a temperature of 30° C., growth set in at once. The fungus which had been kept at the lowest temperature made the fastest growth when it was removed to higher temperature. A temperature ranging from 8° to 10° C. is only slightly inhibitive to growth and when potatoes infected with the organism are stored at this temperature the most rapid decay takes place provided the air is quite moist.

TABLE 6.— Morphogenic and chromogenic influence of factors.

April 14	8.3x3.3 cm., 4x4 cm., fluffy, 5.5 x 5.5 cm., 6.5 x 6.5 cn., 7.5x7.5 cm., very 8x8 cm., very 9.5x9.5 cm., very very pink, white edge, fluffy edge, pink in middle appearance of concentric	In diffuse light, 3 mm, ap-3x3 cm., ap-4x4.5 cm., pow-5.5 x5.5 cm., 6.5 x 6.5 cm., 8.5 x 8.5 cm., 9.5 x 9.5 cm., 9.5 x9.5 cm., 9	c m., 8x9 cm., pow- 8.5 x 9.5 c m., 9.5x9.5 cm., fluffy cover- covering.	2 x 2.5 c m ., 3x3 cm., white, 4x4 cm., white. 5.5 x 5.5 c m., 6.5x7 cm., fluffy, 8.5x8.5 cm., fluffy, sight pink. slight pink, sparse growth	In dark, over H2 .5x.5 cm., 3x3 cm., fuffy 4x4 cm., fluffy, 5.5x5.5 cm., 7x7 cm., pow-8x7.5 cm.,	9.5 x 9.5 c m., 9.5x9.5 cm., pink, pink, fluffy.
Ap	9.5x9.5 pink.	9.5x9.5 pink.	9.5x9.5	8.5x8.5 c pink.	8x7.5 c dery.	9.5x9.5 (fluffy.
April 10	h, very	cm.,	c m.,	., fluffy,	1., pow-	c m.,
Apri	8x8 cm pink, dery.	9.5 x 9.5 pink.	8.5 x 9.5 fluffy ing.	6.5x7 cm pink.	Sx7.5 cm dery.	9.5 x 9.5 pink, f
April 8	m.,very	c m., some- white,	pow-	c m., t	., pow-	
Apr	7.5x7.5 c pink.	8.5 x 8.5 pink, what fluffy.	8x9 cm dery, r	5.5 x 5.5 slight j	8x7.5 cm dery.	1
April 6	c m.,	c m.,	c m.,	white.	Cover	Some plates put into incu-
Apr	6.5 x 6.5 very p	6.5 x 6.5 white,	7.5 x 7.5 fluffy.	4x4 cm.,	dery. Crin., removed.	into bator.
April 5	5 x 5.5 cm., fuffy edge, appearance of concentric	fuffy edge, fuffy edge, secondary whiteness appearing in	cm., fluf- s in	x3 cm., white, powdery, slight pink, sparse growth	cm.,	
Apı	5.5 x 5.5 fluffy appear	5.5 x 5.5 fluffy e second whitened pearing	5.5 x 5.5 copious	3x3 cm., po w slight sparse	5.5 x 5.5 excee fluffy.	***************************************
April 4	fuffy, e edge, middle	a., pow-	em.,	c m .,	fluffy,	
Apr	4x4 cm. whit pinkir	4x4.5 cn dery, pink.	4.4 x 4.5 very p	2 x 2.5 white.	4x4 cm., pink.	
11 3	cm., pink,	d, pow- fluffy ency,	c m.,		fluffy	
April 3	3.3x3.3 very fluffy	3x3 cm pressed dery, tend white.	In dark, tempera5x.5 cm., 3.5x3.5 cm., 44x4.5 cm., 5.5x5.5 cm., 7.5x7.5 ture 25 to 30° C., powdery powdery, very pink. copious fluf- fluffy. humidity 40 to edge, pink, pink, fluffy fluffy.	.5x.5 cm.	SO4, temperature appressed white. 25 to 30° C, in particular appressed white.	
April 1		n., ap-	c m., w dery ge, pink	wth	pressed	
	- 100	t, 3 mr	5x.5	Sligh r gro	2.5x.5	2   5
	t, ten (0 to 40) 1 to 30	ligh re 25 t umidit reent.	30° C 40 t	In dark, tempera-Sight ture 10 to 15° C., humidity 60 per cent.	ver H eratur C., ii	one of these plates removed to incu- bator, humidity 98 per cent.
	unligh ature 3 and 20	iffuse peratu C., bu	ark, te	rk, te 10 to idity	temp o 30°	me of these removed to bator, hun 98 per cent.
	In sunlight, tem perature 30 to 40° C, and 20 to 30° C.	In drem 30° 40 to	In de ture bum	In dan ture humi cent.	In da SO4, 25 t	Some of these plates removed to incubator, humidity 98 per cent.

TABLE 6.—Morphogenic and chromogenic influence of factors—Concluded.

å.	April 14	e m., 9.5 x 9.5 e m., 9.5 x 9.5 e m., 9.5 x 9.5 em., pink, fluffy.  fluffy.	4x3 cm., fluffy 5.5x5.5 cm.,pale 6.5 x 6.5 cm., 8x8 cm., pink, 95 x 9.5 cm., 9.5 x 9.5 cm., 9.5x9.5 cm., 9.5x9.5 cm., very pink. middle.	6.5x6.5 cm., pink, appearing in concentricings, fluffy, on effect	Check started. 5.5x5.5 cm., ex-8.5 x 8.5 cm., 9.5x9.5 cm., fully, tremely fully fully fully fully fully, said white.	No growth.	No growth.
-conclude	April	9.5 x 9.5 cm. pink, fluffy.	9.5 x 9.5 c m., very pink.		8.5 x 8.5 cm, fluffy, pink in	No growth.	No growth.
of lactors	April 8	9.5 x 9.5 cm., pink, fluffy.	9.5 x 9.5 c m., very pink.		5.5x5.5 cm., extremely fluffy and white.	No growth.	
a sultacuce	April 6	7.7 x 7.7 c m., white.	8x8 cm., pink, covered by loose white	mycelium.	Check started.	Removed to No growth.	Removed to Nogrowth.
an one of one	April 5	6.5 x 6.5 cm., very white and fluffy.	6.5 x 6.5 cm., fluffy edge, pinkinmiddle	2.5x2.5 cm., yellow, slimy.		No growth.	No growth.
Care Cereta	April 4	4.5 x 4.5 cm., p o w dery, quite pink, more than in	blue. 5.5x5.5 cm.,pale pink, fluffy.	2x2 cm., yellow, 2x2 cm., yellow, 2.5x2.5 cm., yel-slimy appear-slimy.		No growth.	No growth.
- Fred and one of the state of factors—Concluded,	April 3	Sx3 cm., white, 4.5 x 4.5 cm., 6.5 x 6.5 cm., 7.7 x 7.7 p owdery.  powdery, very white white more than in	4x3 cm., fluffy at edge, white middle.	2x2 cm., yellow, silimy appear-ance.		No growth.	
	April 1					No growth,	No growth.
		In red bell jar, temperature 25 to 30° C.	In blue bell jar, temperature 25 to 30° C.	In dark, humidity 5x.5 cm. 98 per cent, temperature 25 to 27° C.	In dark, humidity 98 per cent, temperature 25 to 27°	In dark, tempera- No growth, No growth, midity 5 to 50 per earl.	In dark, tempera- No growth. No growth. midity 100 per cent.

# TABLE 7.—Influence of lactic acid.

February 12	February 19	February 26	April 3	April 4	April 8
Agar lactic Culture started.	1x1 cm., yellow, slimy.	15x1.5 cm., yellow,slimy	4x4 cm., ex ceedingly pink.	6x6 cm., very pink.	9.5x9.5 cm., very pink.

## Table 8.—Influence of low temperature.

Temperature	April 1	April 27	April 28	May 1	May 8
1° C	Culture started.	Removed to temperature 3.3° C. No growth.		Removed to temperature 22° C.	
−3.9° C	Culture started.	Removed to temperature 3.3° C. No growth.		Removed to temperature 22° C.	Good growth.
−22° C	Culture started.	Removed to temperature 3.3° C. No growth.		Removed to temperature 22° C.	

### HUMIDITY.

The fungus grows best under moist conditions and it is apparent that in most cases the supply of moisture is the determining factor for infection.

#### COLOR.

The color studies were carried on with cultures grown on glucose agar and on potato plugs. In general, the color produced on potato plugs is much more constant than that produced on agar. The general appearance of a colony on agar may show great variation, at times on whole plates and at times only in isolated parts of the same plate. This is undoubtedly due to variation in the condition of the media, to moisture variation, and the like.

The cultures were grown in diffuse light, in the dark, at high and low temperatures, and in analyzed light. The cultures were on agar plates, on potato plugs, and on beef broth. Flask cultures on one per cent glucose and on synthetic media, in tap water and in distilled water were grown in the light and in the dark. Tables 6, 7 and 8 give in detail the observed colors which were noted on a series of cultures. Each series was run in triplicate.

The general color of the fungus when two to three weeks old is a pale pink, sometimes grading over to a flesh pink, Repertoire de Coleurs, page 135, No. 3, and 136, No. 1 (Pl. XXII). In the dark the cultures are more of a flesh pink color than pale pink. Whether the blue or red rays are shut out seems to be imma-

terial. In fact, variation between plates grown under the same condition may be greater than that between plates grown under different conditions. This does not hold for all species of Fusarium by any means, as shown by the Fusarium which we have found on Colorado potatoes. In the light, this fungus assumes a pink color; in the dark, a white, soon followed by a deep blue.

The plates placed in bright light so that the sun struck them during a part of the day, and so that the temperature rose to 30° to 40° C., showed the most intense color, but a slow growth. Here the color very often went over to the salmon tint, page 72 of the Repertoire, especially on the underside of the culture.

The question whether the colony will appear flesh-pink or palepink is largely a matter of secondary germination of the conidia. If these germinate on the original mat and the nutrients become exhausted before the second set of hyphae produce conidia, the mycelium color will tone the conidial color down to a pale pink. Conidia are produced more slowly in such cultures and the mycelial growth predominates. The growth here is rapid and the media are not exhausted completely as the fungus advances; consequently a secondary luxuriant growth takes place over the first layer. In bright light the growth is slower, more conidia are formed, and the mycelium uses up the food completely; consequently less secondary germination takes place and therefore the original pink color is not obscured. The catalytic effect of the sun's rays may have an influence fostering a more complete respiration of the media as the fungus grows along.

The cultures in flasks on liquid media gave contradictory results. The fungus when grown in distilled water and when grown in dark and in light develops a thin mycelium which shows a lilac mauve color, Repertoire, p. 196, No. 1. When grown in tap water the fungus grows very sparingly and develops no color at all. On 1 per cent glucose in light the fungus develops a color. Repertoire, p. 135, No. 3, when two or three weeks old and no surface growth occurs and no conidia are formed. In the dark the growth is very stunted and the color is white. Microscopically no difference, excepting that the fungus grown in the dark showed a very knotted appearance, is apparent. When grown on culture media in the light a very luxuriant growth of white mycelium takes place and no color is developed until the fungus reaches the surface and begins to form a mat of plectenchyma which soon becomes covered with innumerable new conidia. At no time does there develop any color in the mycelium in the medium.

When grown in the natural habitat, the potato away from

light, the fungus is white, but as soon as exposed to light a distinct color develops. In the former case slight conidial formation is the rule, while in the latter great numbers of conidia are formed.

When grown on stems considerable variation in color appeared. When grown on young stems of a tuber grown under irrigation a rich pink color is developed but when grown on stems of a dry land variety the white color predominates. This may in age turn to an ochre tint.

Microscopically no difference can be seen. The cell sap is always of the lilac mauve color in all cultures, both in mycelium and in the spores. By putting the mycelium which has been grown in distilled water into strong mineral acid a red color

often sets in.

The cell sap is always blue. When the growth is weak and no color is developed in the plasma and wall the cell sap color is visible in the mat. If conditions are not favorable for growth, changes in the plasma and walls obscure the cell sap color so that the mycelium is white. Macroscopically the spores are always pink. This color must be located in the plasma or wall and since the nature of the medium determines whether conidia will be formed or not it will also determine whether a pink color will appear or not. The light has an effect in so far as it catalytically influences the medium or in so far as it stunts the growth of the mycelium, causing it to go over into conidial formation. It might do this either by increasing the respiratory activities in the fungus or by inhibiting them.

In the 1 per cent sugar solution in light, the medium must be of such a nature as to produce color and not to cause conidial formation. On beef bouillon (+10) a slight aerial growth took place, few conidia were formed, and a very faint pink color

resulted, so faint that it was scarcely visible.

Color therefore is constant as long as the organism is grown under ordinary constant conditions. The substratum and other environmental factors, especially light, can influence it slightly however. The color is always in the pink category, and no complete changes from red to blue or to yellow, as we have found in the Fusarium from Colorado, are obtained in Fusarium tuberivorum. It is apparent that the nature of the medium and light has something to do with development of color in this organ ism. Which of these is the determining factor we have not been able to ascertain.

If a suspension of old spores is made in water, or when the fungus is grown on liquid media for 6 or 8 weeks, the liquids take on an ochre color. This probably is due to an oil which completely fills the conidia when they are old.

### CONIDIOPHORES.

In Fusarium orthoceras the conidophore is generally only a sterigma-like branch arising laterally from a hypha and cutting off conidia from its end. Sometimes a conidiophore with a tri-sterigmate structure is found. Ordinarily as it is found on potato and as it grows on agar and on potato plugs, Fusarium tuberivorum shows the same structure of conidiophores that Fusarium orthoceras does (Pl. XXVI, fig. 1). In the cold, however, the tri-sterigmate conidiophore appears. On stems and on raw potato plates when quite dry, we find compound conidiophores in which each sterigma-like branch of the first order can branch into three others and thus three or four tiers are formed, presenting a complex compact mass resembling the structure of the conidiophores of Fusarium rubiginosum (Pl. XXVI, figs. 2 and 3).

#### CHLAMYDOSPORES.

In these cultures chlamydospores, which may be borne ter minally or intercalarally, have appeared. Most of these are round and smooth and heavily stored with oil. On beef-bouillon agar and in distilled water the mycelium and spores can be made to fragment into oidia. Each of these cells is quite thick walled, hyaline, full of oil, and capable of germination. Their size generally is  $6-12 \times 8-12$  mu (Pl. XXVII, figs. 1 and 3).

The writers have found single cells and compound cells which are thick walled, other colored, and spiny, only rarely in water and in potato tubes. On a raw potato plate numerous chlamydospores appeared only once in a series of several hundred cultures. The fungus has been grown by others for a year or so in this laboratory and not once did chlamydospores appear during that time (Pl. XXVII, figs. 2, 3, 4, 5, 6, 7, and 8).

It is apparent that with this character we have a variable thing, so variable that under proper conditions the whole fungus can be made to go over into the oidia form and look like a *Cladosporium*. Dry air and low food supply seem to favor the development of these oidia and chlamydospores. The size of the chlamydospore generally is 8-12 x 10-12 mu.

#### SPORE MEASUREMENT.

The measurements of spores taken from various cultures in all stages of development show a surprising uniformity, the only exception appearing in those spores which were grown at low temperatures, they being a trifle wider than the ordinary spores.

On agar the following are the averages:

	Width.	Length.
0-septate	3.2 mu.	8 to 11 mu.
1-septate	3.2 to 5 mu.	11 to 20 mu.
2-septate	3.2 to 5 mu.	12 to 22 mu.
3-septate	4 to 5 mu.	17 to 22 mu.

On potato plugs in the light.

	Width.	Length.
0-septate	3 to 5 mu.	11 to 16 mu.
1-septate	3 to 5 mu.	10 to 18 mu.
2-septate	3 to 5 mu.	16 to 22 mu.

On potato plugs in the dark.

	Width.	Length.
0-septate	2.4 to 3.3 mu.	8 to 13 mu.
1-septate	3.2 to 5 mu.	10 to 20 mu.
2-septate	3.2 to 5 mu.	18 to 25 mu.
3-septate	5 mu.	22 to 25 mu.

The greatest spore length noted was 40 mu. This spore was 3 mu wide. The greatest width noted was 5.8 mu. The great majority of all spores measured is  $5 \times 17-20$  mu.

In measuring spores the diameter of the septum was measured, which gives a reliable basis for width determination. In measuring the length, the length of the segment between the two extremities was measured rather than the length of the arc

of the curvature of the spore.

The spores of Fusarium solani, as to length, compare most closely to those of Fusarium tuberivorum, but they are longer than we find the spores of the latter to be. They also are a trifle wider than the spores of Fusarium tuberivorum. The only other species which could be confused with Fusarium tuberivorum is Fusarium orthoceras. The spores in this species are too narrow, however. Neither Fusarium solani nor Fusarium orthoceras gives the percentages which would make it possible for any one to confuse the three species. The percentages of Fusarium solani according to Appel and Wollenweber are:

1-septate.		٠						8	to 0 per cent.
2-septate.		۰	۰			٠		14	to 0 per cent.
3-septate.			۰					67	to 90 per cent.
4-septate.	 				٠			16	to 0 per cent.
5-septate.								6	to 0 per cent.

For Fusarium orthoceras.

1-septate..... 5 per cent. 2-septate.....Few. 3-septate..... 3 per cent.

4-5-septate ......Few.

For Fusarium tuberivorum, culture 150 days old.

0-septate.....Few. 2+-septate.....Very few.

#### SUMMARY.

We have in Fusarium tuberivorum an interesting organism, both from a morphological and from a physiological point of view. If we consider all species of Fusarium as belonging together, we can look upon Fusarium orthoceras either as the farthest away from its ascomycete ally, if there is any, or as nearest to it. If the contentions that some of the species of Fusarium have been connected with certain Nectria will hold, or that Neocosmospora is closely related, it is apparent that such related species of Fusarium are characterized by the presence of severalseptate curved spores. Then such a form as Fusarium orthoceras would have to be considered the most remote relative. It is possible of course that Fusarium is such a Babel as the genus Gloeosporium and that it is a sort of rendezvous for the ends of several ascomycete forms. If the latter is the case it will be almost useless to try to combine the Fusarium species into a coherent assemblage. On the other hand, if they are related it will be necessary to link only one of these species to an ascomycete form to give all of them their proper place. It is a perplexing state of affairs, however, that the Ascomycetes to which the Fusarium species have often been referred are themselves in about as orderly a state as the Fusarium spcies are.

No matter whether we adopt one or the other view, it seems quite safe to sav that Fusarium tuberivorum shows nicely the transitions by which such forms as Fusarium orthoceras and Fusarium solani or Fusarium theobromae can be connected. Especially is this true since the characters which appear under normal conditions in this fungus can be varied considerably by changing the environment.

Fusarium orthoceras normally shows a preponderating number of non-septate conidia and only a few 3-septate conidia. The spores are not curved and show no decided differentiation of apical and basal cells. Fusarium solani ordinarily shows the curved 3-septate conidium and only rarely the non-septate form, altho this can be changed by putting the fungus under hard conditions. Fusarium solani shows a fair differentiation of its basal

and apical spore cells.

Fusarium tuberivorum shows all the spore forms that have been noted in species of Fusarium. Normally, however, a preponderating number of 1-septate and only a few 3-septate ones occur. The spores show no decided differentiation in their basal and apical cells. Under proper conditions, only non-septate spores appear, and these show no differentiation of their ends. Under other conditions the number of 3-septate spores can be greatly increased and the most typical end differentiation realized.

Fusarium orthoceras shows the simple sort of conidiophore and only at times a simple tri-sterigmate one. Fusarium solani ordinarily shows a compound conidiophore and rarely the simple types. Fusarium tuberivorum usually shows the orthoceras type but can be made to develop the most complex sort of a conidio-

phore built on the tri-sterigmate plan.

Fusarium orthoceras shows typical intercalary and terminal chlamydospores; some species of Fusarium have neither. Ordinarily Fusarium tuberivorum has none but under extreme conditions it can be forced to develop both the terminal and the intercalary kind. As far as size is concerned the spores of Fusarium tuberivorum stand with the smallest of the species of Fusarium, being a little wider than those of Fusarium orthoceras and not so long, and a little longer than those of Fusarium solani and not so wide.

## FUSARIUM TUBERIVORUM SP. N. WILCOX AND LINK.

The following diagnosis is based upon the characters displayed by this fungus when grown on potato plugs in diffuse light at 22° to 27° C.

Conidia not in layers (*Pionnotes* on glucose agar, sporodochia occasionally on old dry tubers of *Solanum tuberosum*). Normal mature conidium fusiform to slightly clavate, slightly bent, no differentiation of terminal cells; 1-septate, generally 3-5x7-20 mu. Few 0-, 2-, 3-septate spores, 2.4-3.3x7-13 mu; limits 4-7 septate, 5.2x40 mu.

Conidial color pale pink, Repertoire des Couleurs des Chrysanthemists, p. 135, No. 3, turning to ochre when very old. Conidiophores simple, sterigma-like, short, occasionally branching in a tri-sterigmate manner.

Subaerial mycelium white, appearing flesh pink, Repertoire, p. 136. No. 1, when mixed with conidia. Chlamydospores appear

rarely in either terminal or intercalary position. Size 6-12 x 8-12 mu.

Occurrence: On tubers of *Solanum tuberosum*, causing a dry rot, and upon dry potato stems.

#### ETIOLOGY.

## TUBER INFECTIONS.

A very large number of inoculation experiments have now been made so that the connection of *Fusarium tuberivorum* with this Nebraska tuber rot is fully established.

The first inoculations were made in 1908 on tubers grown in eastern Nebraska. Puncture inoculations into these tubers gave no evidence of rotting, due no doubt to the comparatively great resistance of these tubers to invasion by this fungus.

Table 9.—Inoculations of western Nebraska tubers made during the winter of 1908-1909.

No. of tubers	No. of inoculations per tuber	Method of inoculating	Humidity	No. of successful inoculations
12 10 8 3	4 4 4 4		60 Over H <sub>2</sub> SO <sub>4</sub> Saturated air 60	48 40 16 Tubers showed a
Check 2	4	Puncture with sterile needle	60	rot.

The tubers kept in a desiccator over sulfuric acid and those kept at the humidity of the laboratory (averaging about 60 per cent during the experiment) gave the largest number of successful inoculations and the greatest amount of rotting. These preliminary experiments clearly established the causal relation of this specific fungus to the dry rot of these tubers.

Extensive inoculation experiments were conducted during the spring of 1910 with several bushels of large healthy Early Ohio and White Ohio tubers grown in Box Butte County. The following types of inoculation were employed in this work:

- 1. Contact with infected tubers.
- 2. Punctures.
  - A. At the "eye."
  - B. At some other point on the surface of the tuber.
- 3. By removal of the "skin" of the tuber.
- 4. Surface infection, with and without media.
  - A. At the stem end of the tuber.
  - B. At a lenticel.

## CONTACT INOCULATION.

On March 26, 1910, a severely rotted Early Ohio tuber was placed in contact with healthy tubers. Similar experiments were made with rotted tubers of the White Ohio potato. It should be stated that, in both cases, the healthy tubers employed had an unbroken surface. On April 26 the tubers were carefully examined and no sign of infection was found in any of the surrounding healthy tubers of either variety.

## EYE PUNCTURES OF EARLY OHIO.

Table 10.—A summary of the results of 102 "eye" punctures, in all of which some medium was applied with the fungus. The greatest amount of rot appeared here on tubers kept at the lower temperature and in the highest relative humidity.

	No. of successful inoculations	Extent of rot	Temperature	Humidity
18 18 18 18 30	18 18 18 18 30	Slight	24 to 27° C. 8 to 10° C. 8 to 10° C.	Over CaCl <sub>2</sub> Over CaCl <sub>2</sub> Saturated air

#### SURFACE PUNCTURES OF EARLY OHIO.

Table 11.—Results of 132 surface puncture inoculations, in all of which medium was applied.

No. of inoculations	No. of successful inoculations	Extent of rot	Temperature	Humidity
18 18 18 78	18 18 18 78	Very slight	25 to 27° C 8 to 10° C	Over CaCl <sub>2</sub> Over CaCl <sub>2</sub>

It will be noted that the rot developed on tubers kept at high or low temperatures and at high or low relative humidity. A greater amount of rotting occurred at the lower temperature with either high or low humidity.

SURFACE INOCULATIONS OF EARLY OHIO TUBERS AFTER REMOVAL OF THE SKIN.



Figs. 1, 2. Inoculation of Early Ohio tubers by removal of the skin. Photographed five weeks after inoculation. Kept in a moist chamber with a small dish of water; the first week at 8° to 10° C. and then at laboratory temperature. Medium applied with the fungus.

Table 12.—Results of inoculation, with and without media, on the surface after the removal of the skin of the tuber.

No. of inoculations	No. of successful inoculations	Extent of rot	Medium	Tempera- ture	Humidity
18 18 18 18 30	0 0 0 11 18	None	Yes Yes	25 to 27° C. 8 to 10° C.	
30	4	2 cm. deep Moderate			Saturated air Saturated air

It is evident in this case that the greatest amount of rotting occurred at high humidity.



Figs. 3, 4. Inoculation of Early Ohio tubers by removal of the skin. Photographed four weeks after inoculation. Kept over CaCl<sub>2</sub>; the first week at 8° to 10° C. and then at laboratory temperature. With medium.

SMEAR INOCULATIONS ON EYE, LENTICEL, OR SURFACE OF EARLY OHIO.

Inoculations, with or without medium, made directly upon the eye, lenticel, or other surface of the tuber gave no infection in any case. Of course in all such cases the surface was unbroken.

Table 13.—Results of a large number of inoculations made March 21, 1910, on Early Ohio tubers which were subsequently kept in an atmosphere having a very high humidity.

Part inoculated		No. of inoculations per tuber	Tempera- ture	No. of successful inoculations	Extent of rot
Eye puncture :	5	6	8 to 10° C.	30	
Eye surface	5 5	6	8 to 10° C.	~0	
Eye surface	5	6	8 to 10° <b>C</b> .	0	
Lenticel surface	-	6	8 to 10° C.	_0	
Lenticel surface	5	6	8 to 10° C.	F 0	
Other than len-					
ticel surface	5	6	8 to 10° C.	0	
Other than len-					
ticel surface	5	6	8 to 10° C.	0	
Epidermis re-	_				
moved	5	6	8 to 10° C.	18	Prominent;
D 11					$\frac{3}{4}$ in. in
Epidermis re-	~		0. 1000		diameter
moved	5	6	8 to 10° C.	4	
Surface punc-	4.0		1000		
ture	10	6	8 to 10° C.	60	



Figs. 5, 6. Inoculation of Early Ohio tubers by removal of the skin. Photographed four weeks after inoculation. Kept in a moist chamber containing a small dish of water; the first week at 8° to 10° C. and then at laboratory temperature. No medium employed.

The last three sets in the above summary were on March 28 removed to moist chambers with CaCl<sub>2</sub> and kept at the temperature of the laboratory.

Table 14.—Summary of inoculations made on March 28, 1910, on Early Ohio tubers subsequently kept in an atmosphere of very high humidity. The tubers were examined on April 27.

Part inoculated	No. of tubers inoculated	No. of inoculations per tuber	Temperature	Extent of rot
Eye puncture Surface puncture Epidermis removed	3 3 3	6 6 6	25 to 27° C. 25 to 27° C. 25 to 27° C.	No rot

Table 15.—Inoculations of Early Ohio tubers made March 29, 1910.

period of the second of the se	Method of inoculation	tubers in-	No. of in- oculations per tuber	Tempera-	No. of successful inocula- tions	Extentf op rot
$\left. egin{array}{c} \operatorname{Over} & \ \operatorname{CaCl}_2 \end{array}  ight.  ight.$	Eye puncture Puncture Epidermis removed.		6 6	8 to 10° C. 8 to 10° C. 8 to 10° C.	18 18	1 cm. deep 1 cm. deep
Satur- ated air	Eye puncture Puncture Epidermis removed.	3 3	6 6	8 to 10° C. 8 to 10° C. 8 to 10° C.	18 18	1.5 cm. dee 1 cm. deep 1 cm. deep
$\left. egin{array}{c} \operatorname{Over} & \ \operatorname{CaCl}_2 \end{array}  ight.  ight.$	Eye puncture Puncture Epidermis removed.	3 3	6 6	24 to 27° C. 24 to 27° C. 24 to 27° C.	18 18	Slight Slight

It is probable that the amount of CaCl<sub>2</sub> employed was not sufficient to give extreme dryness within these chambers.



Figs. 7, 8. Inoculation of Early Ohio tubers by removal of the skin.

Photographed four weeks after inoculation. Kept over CaCl<sub>2</sub>; the first week at 8° to 10° C. and then at laboratory temperature. No medium employed.

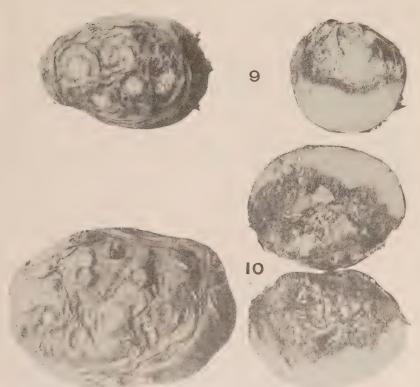
Table 16.—Inoculation experiments with the White Ohio tubers by placing the inoculum on the surface after removing the skin of the tuber.

No. of inoculations	No. of successful inoculations	Medium used	Temperature	Humidity
30 30 6 6 30 30	0 0 0 0 18 4	Yes	25 to 27° C 25 to 27° C 25 to 27° C	Over H <sub>2</sub> SO <sub>4</sub> Saturated air Saturated air

Here again it is evident that the use of medium with the fungus gives a greater number of successful inoculations, particularly at lower temperatures and higher relative humidity.

Table 17.—Unsuccessful inoculation experiments with White Ohio tubers in cases in which the fungus was applied, with and without medium, on the unbroken surface at the places indicated.

Method of inoculation	No. of in- oculations	No. of successful inoculations	Medium used	Temperature	Humidity
Eye surface Eye surface Eye surface Lenticel surface Lenticel surface Lenticel surface Lenticel surface Stem-end surface Stem-end surface Stem-end surface	30 30 30 30 30 60 30 5	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	No Yes No Yes No Yes No Yes No	25 to 27° C. 25 to 27° C. 8 to 10° C. 8 to 10° C. 25 to 27° C. 25 to 27° C. 8 to 10° C. 8 to 10° C. 25 to 27° C. 25 to 27° C. 25 to 27° C. 8 to 10° C.	Saturated air Saturated air



Figs. 9, 10. Inoculation of Early Ohio tubers thru punctures. Photographed four weeks after inoculation. Kept over CaCl<sub>2</sub>; the first week at 8° to 10°C. and then at laboratory temperature.

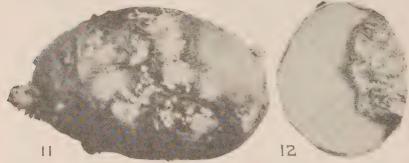
Table 18.—Puncture inoculations made on White Ohio tubers, in all cases medium being applied.

Method of inoculation	No of inocula-	No. of infections	Extent of rot	Temperature	Humidity ;
	tions		·		· - <del></del>
Surface puncture Surface puncture	$\frac{30}{54}$	$\begin{array}{c} 4\\51 \end{array}$	Slight 3 cm. deep		
Surface puncture Surface puncture	6 30	30	Very slight 1 cm. deep		
Eye puncture	30 3	0			
Eye puncture	30	30	1 to 3 cm. deep		

The White Ohio tubers did not prove as susceptible as the Early Ohio tho the rotting was related in the same manner to the factors mentioned in the summaries. At the lower temperatures and with high relative humidity successful inoculations were secured in each case.

Table 19.—Inoculation experiments made with the White Ohio tubers. In all these cases the tubers were subsequently kept in a saturated air at 25° to 27°C. The final examination was made on April 18 with the results indicated.

Method of inoculation		No. of inoculations per tuber	No. of successful inoculations
Eye puncture  Eye surface Stem-end surface  Lenticel surface Surface puncture  Epidermis removed	10	6 6 6 6	0 0 0 0 4 0



Figs. 11, 12. Inoculation of Early Ohio tubers thru punctures. Photographed four weeks after inoculation. Kept in a chamber with a small dish of water; the first week at 8° to 10° C. and then at laboratory temperature.

Table 20.—Inoculation experiments with White Ohio tubers are reported in the following table. Examination made March 18, seven days after inoculation.

Method of inoculation	No. of tubers in- oculated	No. of in- oculations per tuber	Humidity Temperature	Extent of rot
Surface puncture Eye puncture Epidermis removed Surface Surface puncture Eye puncture Epidermis removed	1 1 1 1 1	6 3 6 6 6 3 6	Over H <sub>2</sub> SO <sub>4</sub> 25 to 27° C. Over H <sub>2</sub> SO <sub>4</sub> 25 to 27° C. Over H <sub>2</sub> SO <sub>4</sub> 25 to 27° C. Over H <sub>2</sub> SO <sub>4</sub> 25 to 27° C. Moist chamber 25 to 27° C. Moist chamber 25 to 27° C. Moist chamber 25 to 27° C.	None None None Slight None None



Fig. 13. Inoculation of White Ohio tubers thru punctures. Photographed five weeks after inoculation. Kept in a moist chamber at 25° to 27° C. Only 4 successful infections out of 30 were secured,—the punctures as a rule simply dried out.

Table 21.—Inoculations made March 25 or 28 and examination made on April 25. The tubers were kept at 8° to 10° C. in moist chambers.

					_
Method of inoculation	tubers in-		No. of successful inoculations	Medium used	Extent of rot
Eye puncture	5	6	30	.;;	1 to 3 cm. deep
Eye surface	5	6	0	Yes	
Eve surface	5	6	0	No	
Stem end	5	1	1		
Lenticel	5	6	0	Yes	
Lenticel		6	0	No	
Puncture		6	30	2,0	
Epidermis re-	U	0	00		
moved	5	6	18	Yes	
	Ð	U	10	165	
Epidermis re-	_	0	4	3.7	
moved	5	6	4	No	
Surface not at					
lenticel	5	6	0	Yes	
Surface not at					
lenticel	5	6	0	No	
202202012111111111					



Fig. 14. Comparative extent of the rotting following different types of inoculation. Inoculated March 29.

INOCULATION OF "NEW" TUBERS OF EARLY OHIO,

New potato tubers were obtained at Alliance and inoculated September 3, 1909.

Table 22.—Inoculation of tubers subsequently covered with moist absorbent cotton and left exposed to the ordinary room conditions, the air being usually very dry and warm. The examination was made six weeks after inoculation.

Method of inoculation   No. of   No. of in-   No. of tubers in- oculations   Successful oculated   per tuber   inoculations   Extent of rot	
Epidermis removed 7 2 14 One-half of tuber r	otted
Surface puncture. 13 4 52 One-half of tuber r	otted
Thru scab spots 8 4 0	
Smear 8 4 0	

It will be noted that the simple removal of the skin supplied as suitable a means of entrance for the fungus as did punctures. No infection occurred thru the scab spots nor thru the uninjured surface. The rot developed more slowly than it does in more matured tubers.



Fig 15. Rotted tubers cut open to show the frequent occurrence of a concentric arrangement of the rotted areas.

#### NATURAL FIELD INOCULATIONS.

During 1909, extensive experiments were conducted at Alliance to test the effect of planting rotted seed pieces in the hills. This was designed to throw light upon the question of natural infection under field conditions and the value, if any, of Bordeaux spraying. For this experiment, the following varieties were used:

- (1) Acme.
- (2) Rural New Yorker.
- (3) Early Ohio.
- (4) Red River Valley Ohio.

The Rural New Yorker potatoes employed were purchased in the Lincoln market; the Red River Valley Early Ohio potatoes were purchased in the Alliance market; the Acme potatoes had been grown by W. A. Springer of Alliance, while the Early Ohio potatoes were grown by Wm. Lorance of Alliance.

The arrangement of the rows and the nature of the treatment each received is shown in the following table:

# Potato experiments on the farm of Wm. Lorance near Alliance.

Plot	Row	Variety	
A	1 2 3 4 5 6 7 8	Acme Rural New Yorker—Untreated Lorance's Early Ohio Red River Valley Early Ohio Acme Rural New Yorker—Untreated Lorance's Early Ohio Red River Valley Early Ohio Rural New Yorker—Untreated Rural New Yorker—Untreated Rural New Yorker—Treated with formalin	Without any rot pieces in the hills and not sprayed.
В	1 2 3 4 5 6 7 8 9	Acme Rural New Yorker—Untreated Lorance's Early Ohio Red River Valley Early Ohio Acme Rural New Yorker Untreated Lorance's Early Ohio Red River Valley Early Ohio Rural New Yorker—Untreated Rural New Yorker—Untreated	With "end rot" pieces in the hills and sprayed.
C	1 2 3 4 5 6 7 8 9	Acme Rural New Yorker—Untreated Lorance's Early Ohio Red River Valley Early Ohio Acme Rural New Yorker—Untreated Lorance's Early Ohio Red River Valley Early Ohio Rural New Yorker—Untreated Rural New Yorker—Untreated Rural New Yorker—Treated	With "end rot" pieces in the hills but not sprayed.
D	1   2 3 4 5 6   7 8 9 10	Acme Rural New Yorker - Untreated Lorance's Early Ohio Red River Valley Early Ohio Acme Rural New Yorker—Untreated Lorance's Early Ohio Red River Valley Early Ohio Red River Valley Early Ohio Rural New Yorker—Untreated Rural New Yorker—Untreated Rural New Yorker—Treated with formalin	With "dry rot" pieces in the hills and sprayed.
E		Acme Rural New Yorker — Untreated Lorance's Early Ohio Red River Valley Early Ohio Rural New Yorker — Untreated Lorance's Early Ohio Red River Valley Early Ohio Rural New Yorker — Untreated Rural New Yorker — Untreated Rural New Yorker — Treated with formalin	With "dry rot" pieces in the hills but not sprayed.

Almost one thousand plants were thus exposed to exceedingly favorable conditions for dry-rot infection to occur. Every single

tuber was examined at harvest time and no evidence of dry rot was discovered.

		Per   cent	70	2 09
1909.		10	61	116 75
er of		6	117	82 44
summ		00	132	30
gthe		2	189	39
during	A	9	120	36
unce		rð	163	15
et Alla		4	122 72	38
conducted a		က	175	39
			91 62	74 39
nents		1 2	50 26	34
Table 23.—Data of field experiments conducted at Alliance during the summer of 1909.	Plot	Воw.	Number of marketable tubers  Number with stem-end rot.  Percentage of marketable tubers.	Number of unmarketable tubers

nued.		Per cent	73	2 10
-Conti		10	154	53
-6061		0	123 54	85 34
er of		00	147	39
www.s		2	126	63
g the	B	9	134	35
durin		70	237	333
liance		4	174	
at Al		က	140	15
ncted		2	99	45
cond		-	204	19
Table 23.—Data of field experiments conducted at Alliance during the summer of 1909—Continued	Plot	Вом	Number of marketable tubers.  Number with stem-end rot.  Percentage of marketable tubers.	Number of unmarketable tubers  Number with stem-end rot  Total percentage with stem-end rot

Table 23.—Data of field experiments conducted at Alliance during the summer of 1909—Continued

	10 Per cent	98 31 41 20	0,
	6	132 75	
	00	83 37 30	
	7	125 49  32 12	
O	9	77 46 99 47	
	70	120 28 33 13	
	4	135 63 38 15	
	ල '	137 48 27 5	
	22	131 76 160 107	
	н	177 96 40 12	
Plot	Вож	Number of marketable tubers.  Number with stem-end rot.  Percentage of marketable tubers.  Number of unmarketable tubers.  Number with stem-end rot.	LOTAL Derephtage With stam-and rot

Table 23.—Data of field experiments conducted at Alliance during the summer of 1909—Continued.

Plot						D					
Ком	-	2	3	4	70	9	7	00	6	10	Per cent
Number of marketable tubers. Number with stem-end rot. Percentage of marketable tubers.	113	86	120	102	104	82 24	98	87	124	99	1
Number of unmarketable tubers Number with stem-end rot	36	29	21	16	15	\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	29	32	68	43.	1)
Local percentage with stem-end rot		:						:			47

TABLE 23.—Data of field experiments conducted at Alliance during the summer of 1909—Concluded.

									2003		nonna
Plot						田					
Вом	=	23	ಣ	4	<i>1</i> 0	9	7	00	6	10	Per
Number of marketable tubers Number with stem-end rot. Percentage of marketable tubers	39	82 61	133	187	73	69	103 56	130	74	126	29
Number of unmarketable tubers Number with stem-end rot	16	102		41 24	40	38	24	. 666	120	102	H 0
Total percentage with stem-end rot.	:										52

The data in the above table are of considerable interest since they so clearly show that the dry rot here described cannot be communicated to the underground parts of the potato plant. In the hills in which a badly rotted piece of potato was placed at planting time, as many marketable tubers were produced as in the hills without any such treatment. In fact no dry rot appeared on any of these tubers at harvest time whether dry rot pieces were planted in the hill or not. The planting of tubers affected with stem end rot\* with the seed piece in each hill of plots B and C apparently did not at all increase the percentage of tubers affected with stem end rot or bundle blackening. Previous treatment of the seed potatoes with formalin and subsequent spraying with Bordeaux mixture had no apparent effect upon the crop either as to the total yield or as to the percentage of marketable potatoes.

Moreover, during the seasons of 1909 and 1910 thoro search was made for dry rot on the new tubers. These observations covered ten fields containing a total of about five hundred acres. Not a single affected tuber was found during the summer. Plants and new tubers were secured from various fields during the summer of 1909 and a total of seven hundred and twenty isolations made from tubers and stems but the dry rot fungus was not secured. In one field, however, on August 26, dead potato stalks were found which were entirely rotted and crumbled when disturbed. From these stalks cultures of the fungus were obtained, together with bacteria. During the summer of 1910 in only one field were the plants found to be infected. The organism seems to exist merely as a saprophyte in the field and does not

attack the tubers until they are harvested.

## NATURAL INFECTION WITH DRY ROT.

Potatoes grown in western Nebraska were planted March 1, 1909, in the open bench of the greenhouse. These tubers were quite badly infected with dry rot, but to make certain, thoro infection of the soil, pieces of the rotted tissue were scattered thru the soil with the "seed" pieces. These plants made a good growth and remained green and healthy for twelve weeks. The

<sup>\*</sup>The "stem-end" rot here referred to includes the symptoms of vascular discoloration described and illustrated by Smith and Swingle 1904 and assumed by them and many others to be caused by Fusarium oxysporum (p. 67). During the course of these investigations numerous isolations were made in various ways from a large number of tubers which showed clearly that, when present at all, the Fusarium hyphae were invading diseased tissues. It was found that in many cases saprophytic fungi and bacteria can invade this diseased tissue as readily and frequently as does Fusarium.

new tubers were examined at frequent intervals but no sign of dry rot appeared. After the tops died, forty of the tubers, with the soil still clinging to them, were placed in moist chambers but no dry rot appeared on any of them.

### ARTIFICIAL INFECTION.

During the summer of 1909 at Alliance inoculations with Fusarium tuberivorum were made, in the field, into the growing plant and the newly forming tuber. The first inoculations were made July 29 when the plants were in prime condition. New tubers, three to five centimeters in diameter, were carefully separated out by themselves, wiped clean with cotton, inoculated, and then paper placed around them to prevent contamination. One tuber on each of ten plants was inoculated once. Four inoculations were also made into the stalks of these same plants below the soil level. The soil was carefully replaced over the tubers and around the plant in a manner comparable to the surrounding conditions. The plants were not injured in this way and growth was not retarded. Examinations were frequently made but no infection was found to take place.

In January, 1910, inoculation experiments were carried on under greenhouse conditions. Paraffined baskets were used which were large enough to hold about fifteen kilograms of soil. All paraffined baskets were washed with mercuric chloride and filled with sand which had been autoclaved for one-half hour at 115° C. Potato tubers which were treated with mercuric chloride were simply wiped off with cotton or allowed to dry after a wetting in the solution. The knife used in cutting was heated and cooled between the cutting of the different tubers. In two baskets the seed pieces were taken from healthy tubers and a pure culture of Fusarium tuberivorum placed upon the cut surface of each piece. Sixteen days after planting, the plants were examined. One seed piece was "crumbly" and rotted and the plant was small and worthless. Anoter seed piece was slightly infected but the plant produced was in fair condition of growth. Three other seed pieces were not at all infected and produced strong healthy plants. Twenty-three days after planting neither the new tubers nor the plants showed any infection but all the old seed pieces showed a slight rotting. After the plants had grown for two months in two other baskets they were inoculated just below soil level with Fusarium tuberivorum. No infection resulted.

During the winter of 1911-1912 inoculations of very young shoots were made in the laboratory. None of these were successful. These were made because an apparent infection had taken 3

place in the laboratory in another experiment. Potato tubers had been sterilized with corrosive sublimate solution and then cut into slices and put into sterile petri dishes. Some of the fungus was inoculated into these sections in December just before the Christmas holidays. One of these sections was the bud end of the tuber and the fungus was placed near the terminal bud. These cultures were neglected for several weeks during the holidays and when the researches were again resumed it was found that one of the young shoots that had developed had been overrun by the Fusarium, and the death of the shoot was attributed to the fungus. Later on, a section of one of these slices was used in some inoculation experiments with plants which were covered with bell jars after the pieces had been placed in contact with the stem. The soil and pot had been sterilized, and much to our surprise a luxuriant growth of Rhizoctonia developed in a few days. This showed clearly that the washing of the tuber had not killed all of the organisms in or on the skin of the tuber and reopened the other observations, made on the other slices of the same potato, for further consideration. It is very possible that Rhizoctonia developed during the holidays and killed the shoot, which was subsequently invaded by Fusarium. Other experiments that have been performed with pure cultures and sterilized soil have all been negative in result and therefore this single instance of an apparent infection must be accepted with due reserve.

If this fungus is responsible for the wilt of potato vines at all, it must get into the young underground stems and roots. Manns reports successful inoculations by wounding roots in the presence of the fungus and by growing the potatoes in "sick soil," and says: "The disease came on much more definitely under the sick soil infection than it did where pure artificial cultures were used without incisions or root injury. The great difference between sick soil infection and that from pure cultures or even internal seed infection is that in the use of sick soil the roots are attacked at practically every point, while with pure cultures, or seed internally infected, the fungus attacks only in close proximity to the main root, while most of the secondary roots and the roots and the root hairs remain healthy."

Data from such experiments must, at the best, be very unreliable. "Sick soil," selected at random, may contain a multitude of fungi other than the particular fungus in question. The only reliable way of getting sick soil is to thoroly sterilize the soil and then inoculate it with pure cultures of the organism in question. *Rhizoctonia solani* is reported as an active damping off agent and it may have caused the attacks which are referred

to above as occurring "at practically every point." The present authors' observations of the past summer tend to substantiate this view. The same objections must be raised against all experiments in which wounded plants are planted in non-sterile soil.

These experiments show quite conclusively that *Fusarium tuberivorum* cannot cause a wilt of potato vines by attacking live stems, subterranean or aerial. In spite of the fact that no successful inoculation experiments producing such a wilt with a *Fusarium* have ever been reported, the literature is full of references to such inoculations.

Most workers and reviewers have read inoculation experiments into Smith and Swingle's bulletin tho none are there reported. Most of the publications of the United States Department of Agriculture and the Experiment Stations dealing with the wilt of potatoes consider the causal connection of a Fusarium with a dry rot and wilt as established by this bulletin. These assumptions and statements have even crept into text-books. For example, Duggar 1909 (p. 317) says: "Smith and Swingle have by careful cultural and inoculation experiments demonstrated the causal connection of a Fusarium with these types of disease." In Germany the advocates of the fungus theory of the "Blattrollkrankheit" of the potato have gained most of their support from these assumptions.\*

During the early stages of our investigations of this disease we critically examined the description by Smith and Swingle of a "Dry Rot of Potato due to Fusarium oxysporum" to determine the relationship between the disease there described and the dry rot found in Nebraska. Since no reference to inoculation experiments could be found in their bulletin the senior author wrote Dr. Smith to learn where their inoculation experiments were recorded. Dr. Smith replied that no inoculation experiments whatsoever had been made by the authors.

<sup>\*</sup>Appel and Schlumberger 1911 ((pp. 22, 23): "Da endlich Smith und Swingle Impfversuche beschreiben, bei denen es ihnen gelungen ist, ein der Blattrollkrankheit ganz ähnliches Bild mit ihrem Fusarium oxysporum zu erzeugen, so lag die Erklärung, die Blattrollkrankheit ebenfalls auf ein Fusarium zurückzuführen, sehr nahe."

Further, in a criticism of the enzym explanation of "Blattrollkrankheit" entertained by Sorauer, they say (p. 28): "Wenn aber Sorauer sagt, dass alle Forscher einen anderen Pilz als Fusarium gefunden haben, so hat er dabei eine der wichtigsten Arbeiten übersehen nähmlich die von Smith und Swingle (1904), die eine in ihren Erscheinungen der Blattrollkrankheit mindestens sehr ähnliche Krankheit auf Fusarium oxysporum zurückführen."

Delacroix\* 1906 in a discussion of the black-leg of potatoes due to *Bacillus phytophthorus* expressed doubt as to the causal relation of *Fusarium oxysporum* to the wilt and dry rot described by Smith and Swingle.

COMPARATIVE STUDIES OF FUSARIUM OXYSPORUM AND FUSARIUM TUBERIVORUM.

In order to compare the behavior of the organism isolated from Nebraska tubers showing dry rot with the Fusarium oxysporum assigned by Smith and Swingle as the cause of a wilt and dry rot of the potato, we requested Dr. Smith to send us a culture of the organism with which he worked. Unfortunately these original cultures had died but Dr. Smith requested Mr. W. A. Orton, also of the Bureau of Plant Industry, to send cultures of an organism considered by him to be the same as the one with which Smith and Swingle had previously worked. This organism had been isolated by Orton from tubers, showing dry rot, secured from the Pacific Coast.

A close study of this Fusarium oxysporum and of the Nebraska Fusarium was undertaken in 1909 with special reference to the points which Smith and Swingle had emphasized. The relation of color formation to various kinds of light, to darkness, and to the kind of medium used was studied carefully. The influence of these factors upon the habit of the fungus was observed as well.

The work soon showed that the two organisms were not identical and the Nebraska fungus was set aside as a new species. The results of these studies are found in Tables 24 and 25.

MORPHOGENIC AND CHROMOGENIC INFLUENCE OF ACIDITY OF THE MEDIUM UPON FUSARIUM OXYSPORUM AND FUSARIUM TUBERIVORUM.

The solutions of the acids were made by dissolving the gram molecular weight of the acid in grams in water to make a liter of solution. These solutions are here referred to as N|1 solutions. Different amounts of these solutions were then added to the rice. For example, +100 acetic acid, as employed in the table, means that 5 c.c. of the N|1 solution were added to 50 c.c. of water and that 2 c.c. of this solution were added to 2 grams of rice kernels. The tubes were then autoclaved in a slanting position for 15 minutes at  $110^{\circ}$  C.

<sup>\*</sup>He says (p. 1353): "De plus, j'ai pu constater que le bactérie, qu'il s'agisse du Bacillus solanicola, aussi bien que du Bacillus phytophthorus, est presque toujours accompagnée d'un mycélium de Fusarium; ce mycélium, qui, d'après nos recherches, n'est pas parasite, n'apparait, dans un cas comme dans l'autre, que secondairement. On ne le rencontre pas sur la plante quand la maladie est à son début et j'incline à penser que les cas de parasitisme du Fusarium oxysporum récemment décrits en Amerique par E. F. Smith et D. B. Swingle sur la pomme de terre ne sont peut-être que des faits analogues à ceux que je décris."

The color numbers refer to the Repertoire de Couleurs published by the Société Française des Chrysanthemistes.

014014014

ity.	Color in dark   Colo	136:2 196:1 136:9
	Sclerotia   Col	0+0
ACETIC ACID	Chlamydospores	010
AC	Microconidia	0+0
	Macroconidia	+++
	Growth	+++
	cidity	+6.25 +6.25*

	136:2 196:1 131:2-195: 136:2 136:2 136:2 136:2 136:1 131:2-194: 196:1 131:2	tuberivorum.
2	0+0+011	Fusarium
	01010+ +	* Body type indicates Fusarium oxysporum; black type indicates Fusarium tuberivorun
	0+0+0+ +	ysporum; blac
	+++++++	Fusarium oxi
	+++++0+0000	pe indicates
	+ + 6.25 + 12.5 * * * * * * * * * * * * * * * * * * *	* Body ty

TABLE 24.—Morphogenic and chromogenic influence of acidity—Continued.

	Color in light	131: 1-136: 2 131: 2=194: 4 131: 1-72: 1 131: 2=194: 4 131: 2=194: 4 131: 2=194: 4
	Color in dark	136:2 196:1 136:2 196:1 136:2 196:1
	Sclerotia	0+0+0+
BUILDING ACID	Chlamydospores	01010++
DOG	Microconidia	0+0+0+ +
	Macroconidia	++++++
	Growth	++++++0+0000
	Aeidity	+ + + + + + + + + + + + + + + + + + +

\* Body type indicates Fusarium oxysporum; black type indicates Fusarium tuberivorum.

Table 24.—Morphogenic and chromogenic influence of acidity—Continued.

Color in light	131:1-136:2 131:2 134:1-136:2 134:2-194:4 136:2 131:2-194:4 131:2-194:4 131:2-194:4 131:2-194:4 131:2-194:4 131:2-194:4 131:2-194:4 131:2-136:2	
Color in dark	136:2 196:1 136:2 196:1 136:2 136:2 136:2 136:2 136:2 136:2	11 Dogingoram
Sclerotia	+00+00000++0	Fusarium +
Chlamydospores	100100000 +10	k type indicates
Microconidia	+00+00000++0	sporum: plac
Macroconidia	++++++++++	s rusarium ori
Growth	+++++++++++++++++++++++++++++++++++++++	rype indicates
Acidity	*Body + + + + + + + + + + + + + + + + + + +	Louis th

TABLE 24.—Morphogenic and chromogenic influence of acidity—Continued.

	Color in light	131 131 13 13 13 13 13 13 13 13 13 13 13
	Color in dark	136:2 196:1 136:2 196:1 136:2 136:2 196:1
	Sclerotia	0+0+0+0+
FORMIC ACID	Macroconidia Microconidia Chlamydospores	0111010+
FO	Microconidia	0+0+0+0+
	Macroconidia	   <del>                                   </del>
	Growth	++++++++0000
1	Acidity	125.53 125.53

\* Body type indicates Fusarium oxysporum; black type indicates Fusarium tuberivorum.

TABLE 24.—Morphogenic and chromogenic influence of acidity—Continued.

	Color in light	131:11111111111111111111111111111111111		131:1	131: 2-136: 2	131:1-136:2	139:1
HYDROCHLORIC ACID	Color in dark	136:2 196:1 136:2 196:1 196:1 White		136:2	136:2	136:2 196: 1-128:2	136:2
	Sclerotia	0+0+0+	]	0	to+	010	Fuscasium +
	Chlamydospores	0+0101	LACTIC ACID	0+	-0+	0+0	* Body type indicates Fusarium oxysporum; black type indicates Fusarium turning
	Microconidia	0+0+0+	LA	0+	O+(	 D+C	+ + vsporum: blace
	Macroconidia	+++++		0+	++-	+	Fusarium ox
	Growth	++1+1+00		++-	++-		++ pe indicates
	Acidity	* 0 * 0 * 0 * 0 * 0 * 0 * 0 * 0 * 0 * 0		++52+	*00* +++	+100*	+150* • Body ty

Table 24.—Morphogenic and chromogenic influence of acidity—Continued. MALIC ACID

Color in light	139: 2-136: 2 131: 2-195: 4 136: 2 131: 2-195: 4 139: 1 132: 1-195: 4 132: 1 131: 1 135: 3 135: 3 135: 3 135: 3		131: 1 132: 1=195: 4 135: 3 132: 1=195: 4	
Color in dark	136:2 136:2 136:2 136:2 136:2 136:2 136:2 136:2 136:2 136:2 136:2 136:2		136:2 196:1 136:2 196:1	uberivorum.
Sclerotia	0+0+0+0+0+0+0+0		0100	Fusarium t
Chlamydospores	0+010101010+ 1	NITRIC ACID	0100	ownerson . black two indicates Fusarium tuberinorum
Microconidia	0+0+0+0+0+0+0+	NI	0+0+	peld . munnous
Macroconidia	++++++++++		++++	Daramanim
Growth	++++++++++++		++++00	To do two two to decotor
Acidity	++++++++++++++++++++++++++++++++++++++		+++50 ++100 ++150 +150*	# Dody +

Table 24.—Morphogenic and chromogenic influence of acidity—Continued.

	Color in light	131:1	132:1=195:4	132: 1=195: 4	132:1	135:3	131:1	135:3	131:1	132: 1=179: 1	32.1	132:1	131:1	1 - / 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1
	Color in dark	136:2	196:1 136:2	196:1	196:1	196:1	190:1	136:2	136:2	196:1	128:2 136:2	128:2	136:2 White	iberivorum.
	Selerotia		+0	+0	)+	10	+	OH	-0-	+0	+0	00	00	Fusarium to
CARLIC ACID	Macroconidia Microconidia Chlamydospores	0	10	+0	+	101	PHOSPHORIC ACID	0+	-0-	+0	+0	+0	 )+	type indicates Fusarium oxysporum; black type indicates Fusarium tuberivorum
OAA		0-	+0-	+0-	+	+0+	PHOSP	0+	0-	HO-	+0-	+C	)+	sporum; black
	Macroconidia		<del>  </del>	<del>                                      </del>	+	+++	-	++	++		+	++	-	Fusarium oxy
	Growth	+-+		-+-	<del> </del> 0	+-+-+		++	<del>   </del>	-+	-  -  -	-1	+	pe indicates
	Acidity	++25	***************************************	++100	+150	+ 200 *		* + 25 + 25	*00++	100 + +	+150	+300	+300*	* Body tyl

TABLE 24.—Morphogenic and chromogenic influence of acidity—Concluded.

131: 1-136: 2 132: 1=195: 4 131: 1-136: 2 132: 1=195: 4 1855 3 132 1=195:4 131 1 1=195:4 131 1=136:2 131 1=136:2 132 1 132 1 132 1 135: 3 132: 1=195: 4 131: 1 132: 1=195: 4 131: 1 132: 1 Color in light Color in dark 136:2 196:1 136:2 128:2 136:2 128:2 136:2 196:1 196:1 196:1 196:1 196:1 196:1 136:2 Sclerotia 0+01010+0+ Macroconidia Microconidia Chlamydospores CHECK SULFURIC ACID TARTARIC ACID 0+0+01 Growth Acidity 

\* Body type indicates Fusarium oxysporum; black type indicates Fusarium tuberivorum.

136:2 132: 1-195:4

135:3 132: 1-195:4 CHROMOGENIC INFLUENCE OF LIGHT UPON FUSARIUM OXYSPORUM
AND FUSARIUM TUBERIVORUM.

Solutions of copper sulfate and ammonium hydrate and of potassium bichromate were made in the ordinary manner to provide screens for the development of the blue and the yellow light respectively. Other cultures were placed in the incubator and others exposed to sunlight. In general we note that our Fusarium tuberivorum did not respond quite so readily to the light factor as is reported for Fusarium oxysporum by Smith and Swingle. (See Table 25.)

Table 25.—Showing the resulting color of Fusarium oxysporum and Fusarium tubericorum when grown in various media in blue light, yellow light, sunshine, and darkness for a period of 14 days.

Darbross	Light salmon	White White to pure salmon	White Light salmon	White	White to rosy pink Light, salmon	Light pink Light salmon	White
Sunshine	Salmon	Lilac pink. Light salmon	Delicate pink Salmon	Light salmon Salmon	Rosy pink. Light talmon	Light pink. Salmon	Light pink
Yellow light	Salmon	Light salmon	Salmon	Salmon	Light salmon.	Salmon White	
Blue light	Salmon Lilac bink	Light salmon. Delicate pink	Salmon	Salmon	Light salmon.	Salmon Light pink	
Media	Boiled rice {	Potato agar {	Peptone potato.	Agar banana	Rhubarb	Boiled potato	

The data in the above table and other unpublished data show rather clearly that the organism sent us by Orton as the Fusarium oxysporum of Smith and Swingle differs from the Fusarium tuberivorum in several important particulars. Consequently as early as 1909 the Nebraska organism was considered in this laboratory a new or undescribed species and a tentative name had been given to it.

Smith and Swingle 1904 combined Fusarium solani, Fusarium pestis, Fusarium oxysporum, and other species of this genus. found on potatoes, under the name Fusarium oxysporum.\*

Appel and Wollenweber 1910; examined a culture of Fusarium oxysporum sent them by Orton from Oregon potatoes, and decided that this organism should be called Fusarium orthoceras.

#### RESISTANCE AND SUSCEPTIBILITY.

During the winter of 1911-1912 extensive experiments were conducted to determine the comparative resistance of tubers of different varieties of potatoes to invasion by this fungus. Tho the detailed results of these and many previous similar ones are reserved for future publication, it seems well here to present the general results. From Colorado, thru the kindness of F. L. Fitch. were secured the following sorts:

1. Bliss Triumph

6. Green Mountain

- 2. Burbank
- 3. Early Ohio
- 4. Early Rose
- 5. Gold Coin

- 7. Norton's Beauty
- 8. Pearl
- 9. Raleigh
- 10. Russet
- 11. White Ohio

From Minnesota, thru the kindness of A. R. Kohler of the Minnesota Experiment Station, were secured tubers of the following sorts:

## 1. Early Ohio

## 2. Raleigh

\* Lindau 1908 questions the wisdom of this treatment (p. 470), and says: "Man kann bei den verschiedenen Symptomen, welche die drei beschriebenen Krankheiten haben, zweifelhaft sein, ob man dieser Ansicht beipflichten soll; \* \* \* Man tut wohl am besten, bis durch Kultur und Impfung dieser Punkt geklärt ist, die Verschiedenheit der Krankheiten und ihrer Erreger noch aufrecht zu erhalten."

† (L. c., page 146). "Um völlig sicher zu gehen, dass beide Pilze identisch sind, erbaten wir eine Kultur des F. oxysporum der Verfasser. Wir erhielten eine solche durch die Güte des Herrn E. F. Smith, zwar nicht eine Originalkultur, sondern eine in Oregon von Herrn W. A. Orton aus 'Dry rot'—kranken Kartoffeln gezüchtete Art. Die letztere war im Reagensglase verschlossen Herrn Smith nach Washington zur Diagnostizierung übersandt worden, wurde aber den 27. IV. 1909 uneröffnet nach Deutschland weitergeschickt als vermutlich F. oxysporum Smith und Swingle."

Sound tubers, previously sterilized with corrosive sublimate, were placed in contact with thoroly rotted tubers. In not a single case did infection of the sound tubers result.

Inoculum was placed over lenticels and over the "eyes" but no infection resulted in any of the varieties. In no case did in-

fection occur.

Sound tubers were sterilized with corrosive sublimate and inoculated thru a wound. None of the varieties mentioned above escaped infection but there was evident some slight difference as to the extent of the rotting in the different sorts. These results fully confirm those previously secured. (See Plates I to XII.)

#### OTHER INOCULATIONS.

In the winter and spring of 1911-1912 experiments were conducted to determine the comparative resistance of aerial stems of various varieties of potatoes to the invasion of Fusarium tuberivorum. The following sorts were obtained from Colorado thru the kindness of F. L. Fitch of the Colorado Experiment Station:

1. Raleigh

2. Early Rose

3. Burbank

4. White Ohio

5. Early Ohio6. Russet

7. Bliss Triumph

8. Norton's Beauty

9. Gold Coin

10. Pearl

11. Green Mountain

12. Spaulding's Rose

13. Beauty of Hebron

14. Snowflake

From Minnesota, thru the kindness of A. R. Kohler, were secured tubers of the following sorts:

## 1. Raleigh

## 2. Early Ohio

These tubers, previously sterilized by soaking in corrosive sublimate solution (1 to 1000) for one-half hour, were planted in soil which had been previously sterilized by steaming in pots which had been sterilized with formalin solution. These pots were kept in the greenhouse and watered, as need arose, with distilled water.

When the plants had attained a height of about 30 to 35 cm. inoculation was attempted. In each pot some stems were wounded quite deeply with a sterile scalpel before the inoculum was applied. This inoculum consisted of a mixture of spores, hyphae, and glucose agar. Other stems were merely smeared with the inoculum without previous wounding. Four stems in each pot were used for the experiment, two with wounds and two without wounds. The humidity of the atmosphere in the green-

house was kept as high as possible during the inoculation and immediately following it so that conditions for germination of spores and hyphal development might be as favorable as possible.

No infections resulted from the 64 inoculations. In many cases the fungus flourished on the stems and in the wounds for a considerable period. On one stem a slight browning developed but no fungus developed in the stem. The wounded stems promptly developed wound cork which very often sloughed off with the inoculum tho in many the inoculum persisted until the death of the stems. In several cases the fungus renewed its growth as soon as the stems died, but in not one did the fungus enter the stem and cause a wilt. Several stems were bruised so severely at the time of inoculation that they soon toppled over and either lived on in this prostrate position or died by strangulation. Such dead stems were promptly overrun by the fungus.

Following these failures in producing the wilt of the stems, the experiments were repeated on younger stems of the same

plants. No infections could be obtained.

Some vines of the Pearl variety were bruised with a sterile scalpel by cutting out a piece of the bark deep enough to expose the wood. Inoculum was smeared into these wounds, which were then covered with moistened sterile cotton which was kept moist for a week with sterile water. Wound cork was formed both in the inoculated and control stems but no infection set in. Twelve inoculations were made and all were unsuccessful in producing any wilt. Upon death of the stems the fungus lived upon them saprophytically.

These experiments show quite conclusively that the fungus cannot infect aerial, living stems of the potato, either thru wounds or thru the epidermis. The experiments also show that the fungus does attack stems as soon as they are dying or dead.

(See Pls. XII to XXI.)

Appel and Schlumberger 1911 refer to certain infection experiments with the Fusarium oxysporum of Smith and Swingle

conducted by Köck 1909.\*

We have previously discussed certain results which show clearly considerable variation in the susceptibility of the tubers of different varieties to the dry rot. The explanation for this may be found in one of several factors which may here be mentioned. The short growing season for potatoes in western Nebraska and particularly the frequent occurrence of killing

<sup>\*</sup> They say (p. 24): "Auffallend ist bei seinen Versuchen, dass auch die Infectionen mit Originalkulturen des Erregers des dry rot, Fusarium oxysporum, nicht gelangen. Diese Impversuche fussen im wesentlichen auf der ursprünglichen Annahme Appels, dass die Eingangspforte für die erste Infektion der unterirdische Teil des Stengels sei."

frost before the vines have fully matured may be sufficient to account for the presence of a thinner and more readily penetrable skin than might be found under other circumstances. A histological examination of tubers from various sources tends to support this explanation tho further studies are required to

put the matter beyond doubt.

The failure of stem inoculations, or at least the very weak infection there found, would lead one to suppose that there was some substance in the stem, not found in the tuber, which tended to render the stem more immune against invasion by this fungus. The further fact that young tubers are infected neither in the field nor readily in the laboratory is still further evidence of this same sort. The distribution of solanin in the different parts of the potato plant is known to agree fairly well with the observed facts of inoculation. The exact relationship of solanin distribution and its relative abundance in different parts of the same variety of potato and in different varieties is now being investigated in this laboratory.

It should be stated that it has been shown by one of our colleagues\* that the continuous growing of potatoes in the hot soil of eastern Nebraska apparently tends to result in weakened vigor. This tendency may be largely overcome thru the use of a mulch or the importation of seed from a cooler climate. Just what, if any, effect these conditions may have upon the development of the skin or the distribution of solanin remains to be

determined.

#### METHODS OF CONTROL.

The following experiments were conducted to learn if any method of treatment might be employed by which the rotting of the tubers during storage might be largely or entirely prevented. Arrangements were made to conduct these experiments in the cement potato cellar of Lincoln Davis near Gordon, Nebraska, and to him we are indebted for his enthusiastic interest and assistance in this phase of the investigation. A total of eighty-one bushels were employed in the experiment and these were divided into ten lots of about eight bushels each and treated before being placed in storage, as follows:

- 1. Flowers of sulfur. The tubers were rolled in this powder so that their entire surface was covered.
- 2. Air-slaked lime. The tubers were thoroly dusted over with this powder.

<sup>\*</sup> Emerson, R. A., Bul. 97 Nebr. Agr. Exp. Station.

- 3. Lime and sulfur. The tubers were dusted over with a powder composed of three parts of air-slaked lime and one part of the flowers of sulfur.
- 4. Lime-sulfur wash. This was made by boiling in the usual manner five pounds of sulfur, five pounds of lime, and fifteen gallons of water. After treatment the potatoes were dried before being placed in the storage bin.
- 5. Formic aldehyde solution. The tubers were placed in sacks and dipped for two hours in a solution of one pint of 40 per cent formic aldehyde solution (often called "formalin") in a barrel of water. The tubers were then dried before being placed in storage.
- 6. Formic aldehyde vapor. The tubers were exposed to the vapors generated by twenty-three ounces of potassium permanganate placed in three pints of a 40 per cent solution of formic aldehyde (formalin) to each one thousand cubic feet of space.
- 7 Check. These tubers were not treated at all.
- 8. Check. These tubers were wounded by walking over them before they were placed in storage.

The tubers were placed in bins two feet wide and five feet long and were about three feet deep in each of these bins. The bins had previously been thoroly disinfected with formic aldehyde solution. Treatment was applied and the potatoes were stored October 1 and the counts were made the following April 25. The results are shown in Table 26.

In the first eight lots the World's Fair potato was employed, while for two of the checks smaller lots of the Blue Victor and the Early Ohio were employed.

Table 26.—Results of experiments in the control of dry rot.

Treatment	Total number of tubers	Number rotted	Per cent rotted
1. Flowers of sulfur 2. Lime 3. Lime and flowers of sulfur wash 5. Formalin dip 6. Formalin vapor 7. Check—not bruised 8. Check bruised 9. Check—Early Ohio	1393 1208 1420 1221 1459 1121 1262	133 227 291 45 60 67 182 397 316	12.00 16.08 24.08 3.16 4.09 4.59 16.23 31.46 31.60
10. Check—Blue Victor	641	67	10.45

This experiment has clearly demonstrated that dry rot during storage may be held in check thru treatment of the tubers before

being placed in the storage cellars. For this purpose the best results were secured thru the use of either formalin dip, formalin vapor, or the lime-sulfur wash. Not only did the tubers in these lots show a very small percentage of dry rot but the tubers themselves were in excellent condition otherwise when removed in April. The storage time, it should be remembered, employed in this experiment is longer than would ordinarily be employed by the average farmer and this gave the treatments a severe test. Under ordinary farm conditions the development of the formalin vapors is not easily secured and therefore we would particularly recommend the use of the formalin dip as the easiest method to employ and one that should give excellent results in practice.

In connection with the above experiment a quantity of the World's Fair tubers were selected that showed bundle blackening or stem-end rot. The stem ends of these tubers were cut away till none of the discolored tissue remained. These tubers were then stored for the same length of time with the lots above mentioned with the following results:

Table 27.—Effect of removal of the discolored stem ends.

Total number of tubers	Number show- ing stem-end rot	Per cent show- ing stem-end rot	Number show- ing dry rot	Per cent show- ing dry rot	
1129	24	2.14	426	37.73	

This experiment clearly shows that the removal of the stem end of tubers showing characteristic stem-end rot tends thru wounding the tubers to greatly increase the amount of rotting due to dry rot and hence could not be practiced with safety in the control of the bundle blackening.

#### SUMMARY AND CONCLUSIONS

1. The dry rot of the Irish potato tuber herein described is primarily a disease of mature tubers.

2. This rot is of great economic importance thru the serious

destruction of the tubers while in winter storage.

3. This dry rot has been shown by numerous infection experiments to be due to a new species of *Fusarium*, herein described under the name *Fusarium tuberivorum* sp. n. Wilcox and Link.

4. This fungus has been shown to be incapable of infecting

any other part of the potato plant than the tuber.

5. This fungus cannot invade the tuber until it has practically attained maturity and then only thru wounds. Infection thru scab spots, lenticels, or the eye seems to be impossible. During

the winter, the tuber is destroyed more rapidly by this dry rot than at harvest time.

6. It has been found possible to greatly reduce the amount of dry rot by proper fungicidal treatment before the tubers go into storage. Wounded tubers had best not be stored with sound tubers.

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of the tuber and the sporodochia-like pustules which have broken thru the surface of the rotted area. Photographed six weeks after inoculation. Natural size.



Plate 11. Languaginal section of a rolled tuber. Bacteria have here entered and caused a wet rot and brown discolaration in advance of the dry set due to Fauerian. Photographed six weeks after insculation Natural size.



face. No exterior signs of the fungus are evident. When the tubers are kept in a dry place the fungus grows entirely within the tuber. Photographed six weeks after inoculation.



Plate IV. The upper tubers show the result of puncture inoculation; the lower ones show the failure of eye surface application of the inoculum.



Plate V. Upper tuber inoculated and lower punctured with a sterile needle. Both were then cut open thru the puncture. Photographed six weeks after inoculation.



Plate VI. Characteristic development of dry rot following inoculation thru a puncture. The tubers were cut open to show the details and extent of the rotting.



Plate VII. Development of dry rot in tubers kept in the refrigerator in a humid atmosphere. The longer tuber is the Burbank; the shorter one is Early Ohio. Photographed four weeks after inoculation.



than VIII Tuber showing a sight amount of rettler at the inoculation points. These sides in the refreguence in a h mid atmosphere. Photographed four weeks after inoculation.



Plate IX. Photographs of tubers cut open six weeks after inoculation This shows an extensive rotting of the tubers. No. 1 is the Pearl and No. 2 is the Burbank.

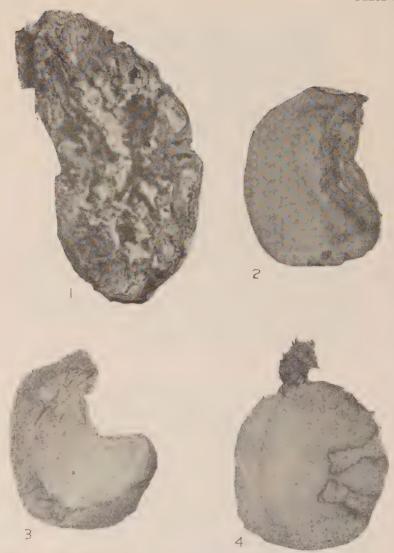


Plate X. Fig. 1 shows the final stage of dry rot in which the entire tuber is converted into a dry mass of tissue remnants, starch, hyphae, and spores. This tuber was kept in the laboratory on a table after inoculation. Fig. 2 shows the characteristic appearance of the rotted region. This is an Early Ohio tuber kept in the refrigerator in humid air. Fig. 3 shows the exterior of the tuber shown in Fig. 2. Here is a copious development of the fungus on the outside of the tuber. Fig. 4 shows slight development of the rot in a deep inoculation puncture. All photographed six weeks after inoculation.

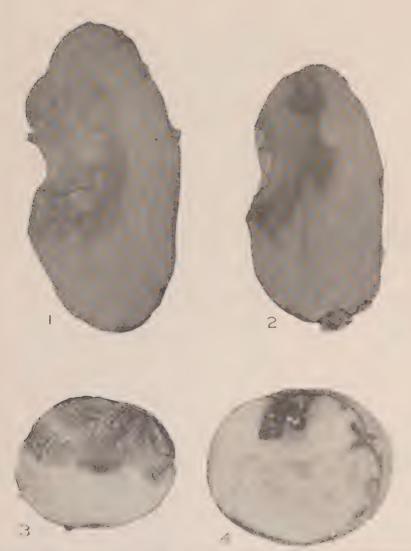


Plate XI. Sections showing characteristic bacterial contamination following inoculation. Early Ohio tubers. The tissue is soft and discolored because of this bacterial invasion in advance of the Fusarium. Photographed six weeks after inoculation.



Plate XII. Inoculation of stems, near the surface of the ground. The fungus failed to make any growth in the stem until the two stems which had been cut deeply in the inoculation fell over and began to dry out. Photographed two weeks after inoculation.



Plate XIII. Shows the appearance of the inoculated  $s_1$  ets eight days after inoculation. The stems are quite normal and healthy in appearance.



Plate XIV—Further inoculations of stems. One of these has broken over as a result of the inoculation wound without any extension of the fungus into the tosues of its stem. The drooping of the foliage here is due to the fact that this plant was accidently "nipped" by frost in moving it from the laboratory to the greenhouse. In spite of this frost injury these plants escaped infection and regained their normal appearance and vigor. Photographed two weeks after inoculation.



Plate XV. Inoculated stems. These wounds, after inoculation, were covered with moist cotton. In spite of this the fungus did not grow into the tissues of the stem but, as the result of cork formation, the inoculum was simply sloughed off. Photographed two weeks after inoculation.



Plate XVI. The inoculated stems here have broken over as the result of the inoculation wound. In this partially dried condition of the stems they are attacked by this fungus. Photographed two weeks after inoculation.



about the wound that the upper part wilted. The fungus did not ad-



Plate XVIII. These inoculated stems have fallen over for the reason stated under Plate XVII. The fungus did not invade the stolons and tubers the the subaerial stem had died. Photographed two weeks after inoculation.



Plate XIX. The foliage after modulation of the stems was severely attacked by thrips. In spite of this minute the finners made no growth till the plant had died down and then the subterranean parts were not infected. Photographed six weeks after inoculation.



Plate XX. Shows the perfectly healthy foliage on stems eight days following the inoculation



Plate XXI. Inoculated stems still showing, two weeks after inoculation, perfectly healthy foliage. The fungus had not extended into the tissues of the stem. The inoculum is being sloughed off thru the formation of wound cork.

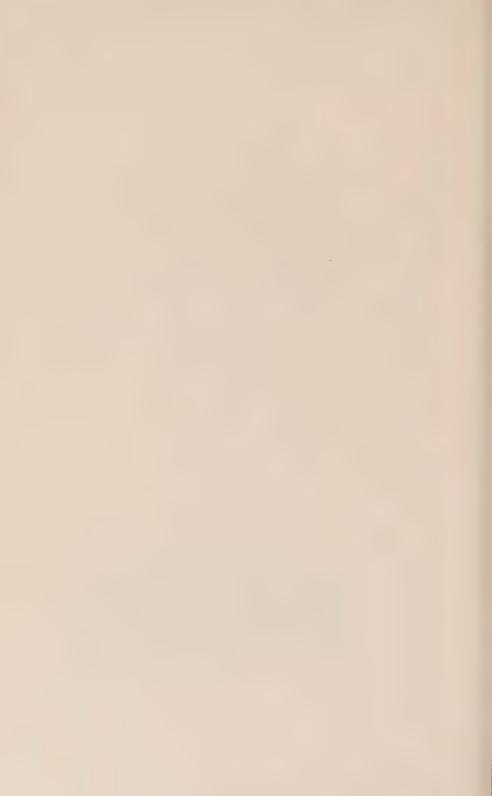


Plate XXII. A culture of Fusarium tuberivorum sp. n., showing its natural color and general appearance. This was grown in glucose agar and was two weeks old when photographed.



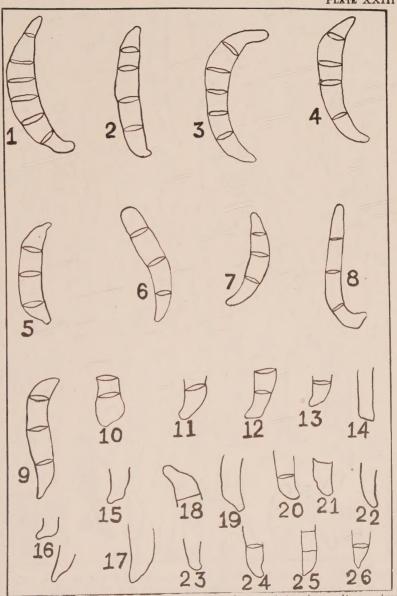


Plate XXIII. Figures 1 to 9 show spores from potato plug cultures, two weeks old, grown in the refrigerator at a temperature of 8° to 10° C. × 1000. Figures 10 to 17 and 19 to 26 show various degrees of development of the basal cell of spores from cultures on potato plugs, two weeks old, in a refrigerator at a temperature of 8° to 10° C. × 1000. Figure 18 shows the apical cell of a spore from the same culture as figures 10 to 17. × 1000.

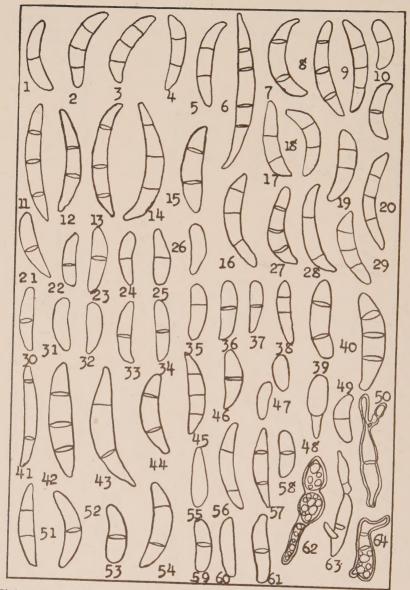


Plate XXIV. Figures 1 to 20 show spores from a potato plug culture, three weeks old. They are all descendants of a single 3-septate spore. × 1000. Figures 20 to 26, 30 to 38 show spores from raw potato culture two weeks old. × 1000. Figures 39 to 45 show spores from potato plug culture grown in diffuse light, three weeks old. × 1000. Figures 46 to 49, 53, 55 to 58, and 60 show spores from a potato selected at random. × 1000. Figures 50, 63 to 64 show spores from beef bouillon agar, fragmenting into oidia. One of these cells has germinated. × 1000.

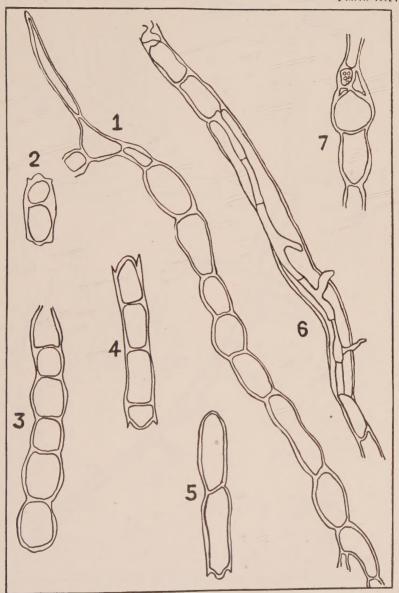


Plate XXV. Figure 1 shows a hypha from culture on beef bouillon agar. Figure 2 shows a spore-like fragment of the mycelium with the peculiar end structure. Figures 3 to 5 show hyphae from a culture on beef bouillon agar. Figure 6 shows the growth of a hypha into empty cells and its subsequent branching. All figures × 1000.

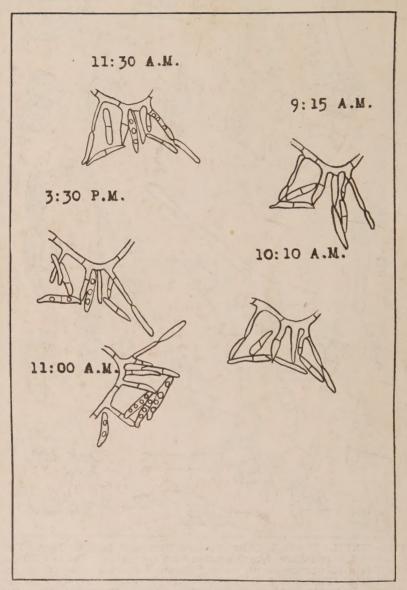


Plate XXVIII. Observations of conidiophores cutting off spores from 9:15 A. M., April 22, till 11 A. M., April 23.  $\times$  540.